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SELECTIVE MATING AS A CAUSE OF GENE FREQUENCY CHANGES IN LABORATORY POPULATIONS OF *DROSOPHILA MELANOGASTER* [1]

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GENERAL CONSIDERATIONS

The results of mating experiments with several sex-linked mutants and the wild type of *Drosophila melanogaster* have already been published (Merrell, 1949a). If the mating success of the various types of flies is assumed to be the same in fairly large populations as it was in the mating experiments when small numbers were used, the changes in gene frequency due to selective mating in the populations can be calculated. Comparison of the predicted changes with the changes observed in populations of these flies will then reveal what proportion of the total change in gene frequency can be attributed to selective mating.

The analysis is clearest when individuals with just one of the sex-linked recessive genes are in competition with others bearing the wild type allele from the Lausanne Special stock. (See Merrell, 1949a for further description of the stocks used.) The genes, yellow, cut, and raspberry, tended to make the recessive males less successful in mating than the wild type. Except for homozygous cut females, which mated only one-half as often as the heterozygous females, the various female types did not differ significantly in mating behavior from the wild type. Forked had no apparent effect on the mating success of either sex.

Therefore, except in the case of forked, selective mating is expected to lead to a reduction in frequency of the mutant gene and to the elimination of the aberrant phenotype. The loss of these sex-linked genes should proceed more rapidly than the loss of autosomal genes comparable in effect. The autosomal recessives can be carried by heterozygotes of both sexes, but a sex-linked recessive is always exposed to selection in the males. Thus, the population will be brought quite rapidly toward the wild type. If mutant males appear as offspring of heterozygous females, their lack of mating success will lead to further reduction in the frequency of the gene. Furthermore, in order for females homozygous for a recessive sex-linked gene to appear, the father must be of the mutant type. Lack of success by the mutant males means that very few recessive fe-

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TABLE 1

Male competitors	Ratio of success	M_m (approx.)
y:+	11:130	0.1
ct:+	9:64	0.15
ras:+	39:79	0.5
f:+	63:52	1.0

males will appear. If the logical assumption is made that the wild type flies represent the better adapted phenotype, then the effect of selective mating is to maintain the population closer to the adaptive norm than would be possible if mating were at random. The frequency of mutant types in collections from wild populations is usually low even though the number of hidden recessives may be large (see Spencer, 1947 for discussion). Selective mating, as well as the lower viability of the recessive types, may be a contributing factor to the high proportion of phenotypically wild type flies.

The rate of loss of the mutants may be calculated with the use of the mating ratios (Merrell, 1949a, 1950) given in table 1. If equal numbers of yellow and wild type males were present, the yellow males would fertilize only 0.1 as many females as the wild type males. As the percentage of yellow males decreases, it is possible that their relative success becomes even less than 0.1 due to crowding. However, since no estimate of this possible crowding effect was available, it was not taken into consideration in making the calculations. Among the females, all were assumed to mate with equal frequency except the homozygous cut females, for whom M_t equaled 0.5 (59:116). With these values for the mating ratios and the equations published in a previous paper

TABLE 2. *Competition in populations with yellow and wild type alleles**

Generation or count	Day	Number of populations	*N*	% Males	q_m (obs.)	q_m (calc.)	q_f^2 (obs.)	q_f^2 (calc.)
0	0	16	400	80.0	.500	.500	—	—
1	30	16	1229	44.3	.333	.500	.022	.045
2	60	16	2172	45.3	.227	.295	.029	.027
3	90	16	1870	42.4	.134	.193	.008	.008
4	120	16	892	45.3	.077	.117	—	.003
5	150	15	1615	49.2	.028	.070	—	.001
6	180	12	733	47.4	.006	.042	—	.000
7						.024		.000

* Observed data from Ludwin (1951).
q_m—proportion of males with the mutant.
q_f^2—proportion of females homozygous for the mutant.

TABLE 3. *Competition in populations with cut and wild type alleles**

Generation or count	Day	Number of populations	*N*	% Males	q_m (obs.)	q_m (calc.)	q_f^2 (obs.)	q_f^2 (calc.)
0	0	9	225	80.0	.500	.500	—	—
1	30	9	451	42.1	.321	.500	.007	.065
2	60	9	635	41.9	.132	.292	.003	.038
3	90	9	830	39.7	.088	.196	.002	.011
4	120	9	468	49.5	.013	.122	—	.004
5	150	9	833	49.6	—	.077	—	.002
6	180	9	784	46.7	.003	.048	—	.001
7						.030		.000

* Observed data from Ludwin (1951).

TABLE 4. *Competition in populations with raspberry and wild type alleles*

Generation or count	Day	Number of populations	N	% Males	q_m (obs.)	q_m (calc.)	q_f^2 (obs.)	q_f^2 (calc.)
0	0	11	165	66.7	.500	.500	—	—
1	36	11	1307	39.7	.443	.500	.099	.167
2	66	11	2742	42.4	.350	.417	.078	.139
3	104	11	1856	48.5	.260	.375	.060	.099
4	134	11	1589	53.5	.282	.319	.053	.074
5	166	11	1939	49.4	.241	.275	.032	.052
6	204	11	1245	49.5	.178	.232	.030	.037
7	257	7*	689	51.8	.134	.196	.006	.026
8	324	7	856	40.0	.061	.164	.006	.018
9	356	7	688	48.5	.063	.136	—	.012
10	388	7	503	39.2	.015	.113	.006	.008
11	418	7	488	40.6	.081	.093	—	.006
12	458	7	305	51.8	.051	.076	—	.004
13	489	6	450	46.0	.024	.063	—	.003
14	528	5	191	48.2	.033	.051	—	.002
15	555	5	294	38.1	.009	.042	—	.001
16	589	5	241	32.8	.013	.034	—	.001
17	621	5	341	54.8	.005	.028	—	—
18	656	4	246	38.2	.011	.022	—	—
19	689	4	231	49.8	—	.018	—	—
20	720	3	238	60.5	.014	.015	—	—
21	763	2	169	37.3	.016	.012	—	—
22	807	2	137	43.1	.017	.010	—	—
23	843	2	194	42.8	—	.008	—	—
24	880	1	79	57.0	.044	.006	—	—
25	920	1	87	23.0	—	.005	—	—
26	953	1	130	59.2	—	.004	—	—
27	986	1	68	41.2	—	.003	—	—
28	1032	1	64	67.2	.023	.002	—	—
29	1078	1	147	64.6	.063	.002	—	—
30	1193	1	73	57.5	.024	.002	—	—
31	1259	1	106	47.2	—	.001	—	—
32	1318	1	46	41.3	—	—	—	—
33	1351	1	134	62.7	.048	—	—	—

* Four populations lost.

(Merrell, 1950), the changes in frequency of the various genes have been calculated for tables 2, 3, and 4 and plotted in figures 1, 2, and 3. An added refinement might have been made in the calculations by taking into account the unbalanced sex ratio in the populations. However, the percentage of males is quite variable from one count to the next so that the accuracy to be gained is relatively slight. Furthermore, analysis of the changes in gene frequency for each sex separately reveals more clearly the way in which the changes occur.

EXPERIMENTAL METHODS AND RESULTS

The populations containing the mutants and their wild type alleles were maintained in population bottles (Reed and Reed, 1948). The data for the populations are given in tables 2, 3, 4, and 5 and in figures 1, 2, 3, and 4. The results for the yellow and cut laboratory populations are from Ludwin (1951). In all of the experiments, the populations were started with equal numbers of mutant and wild type males and with only heterozygous females. The initial gene frequency, therefore, was 0.5. For the raspberry and forked populations, five males each of the

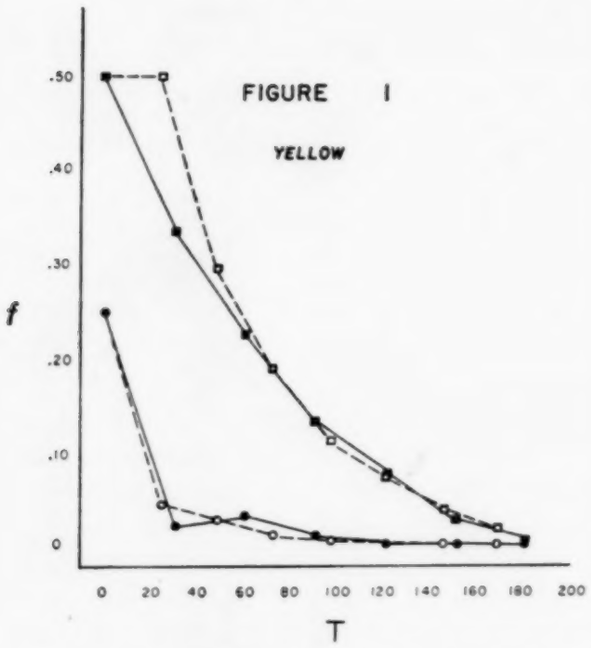

FIG. 1. Competition between yellow and wild type. T is the time in days; f the frequency of mutant males (□ calculated, ■ observed) and mutant females (○ calculated, ● observed).

mutant and the wild type and five heterozygous virgin females made up a starting population. These populations were maintained at $20 \pm 1°$ C. in a constantly lighted room on a standard corn meal, agar, corn syrup, and yeast medium. Samples of the populations were attracted by light into a vial held over the hole in the rubber hose which is ordinarily plugged with cotton, and then etherized and counted.

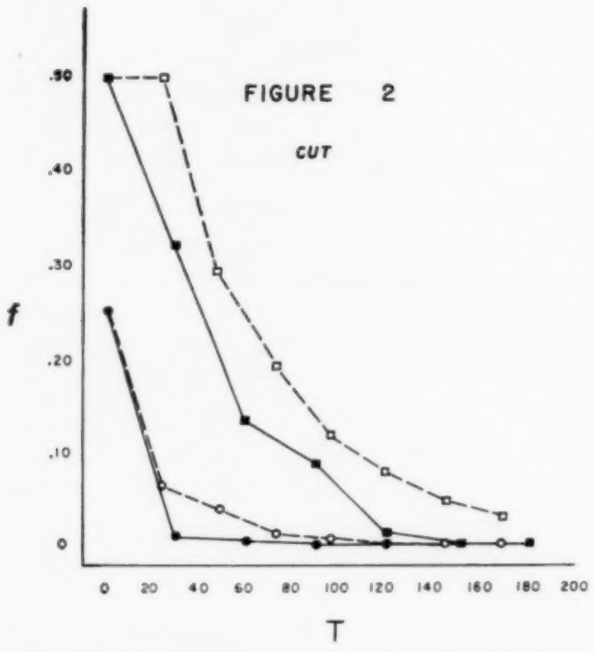

FIG. 2. Competition between cut and wild type.

For each mutant at least nine population bottles were started. Each unit, of course, contains an independent population. However, since the course of events was similar in all of the units with the same mutant, it is possible to treat them as a single large population rather than nine separate ones. But when a mutant is eliminated entirely from a unit, that population differs qualitatively from the units in which both alleles are still present. At that point, some error is involved in the lumping of the data. If all units are included throughout, the group of populations is not homogeneous, and a false impression of gradual loss of the gene is obtained. If only those units still showing the mutant phenotype are included, some populations still carrying the mutant in heterozygous females may be excluded, and the gene may appear to be more frequent than it actually is. The errors introduced are not great, however, since the decrease in gene frequency in the units was regular and gradual. For purposes of uniformity it was decided arbitrarily to consider the mutant gene lost from any population in which it had not been counted over a period of six months and to drop the population from the data at the end of that time. Some of these populations were followed for additional periods of up to a year, and in no instance were mutant individuals later found. However, this procedure is still somewhat misleading in that figures 3 and 4 show gene frequencies at the ends of the curves based on only the 2 or 3 populations still retaining the gene and thus the values, in terms of what has happened in all the populations, tend to be high. (The data from table 4 are plotted only through the 843rd day in figure 3 since beyond that time only a single population still carried raspberry.)

The results show that the frequency of yellow and cut dropped very rapidly until

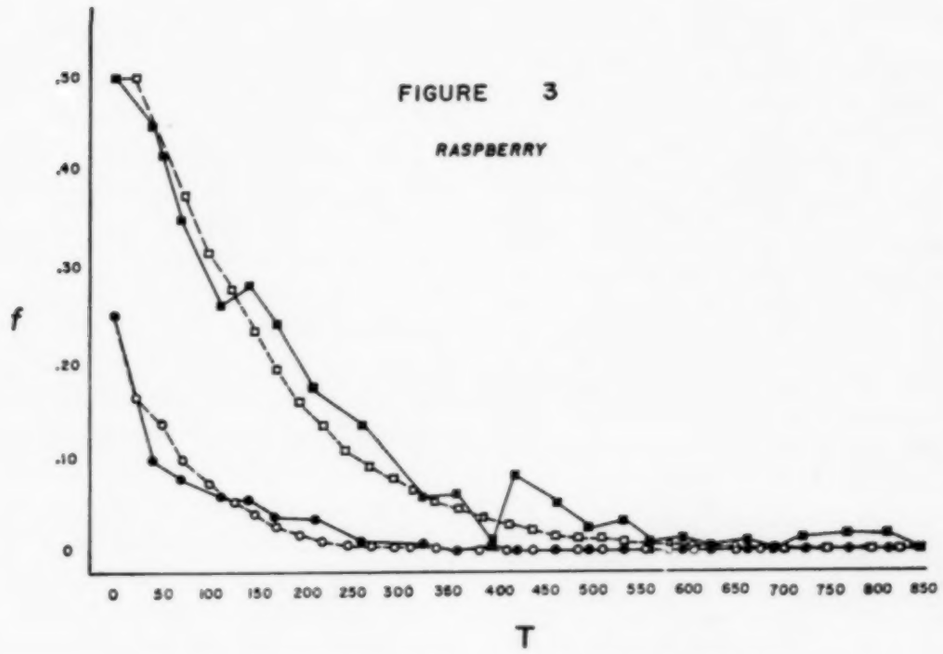

FIG. 3. Competition between raspberry and wild type.

practically zero at the end of six months for yellow and four months for cut. These genes had the greatest effect on male mating ability. Raspberry, which had less effect on mating, and forked, which had none, showed a more gradual rate of elimination. Ludwin concluded that both raspberry and forked reached equilibria, *ras* at 0.5 and *f* at 0.33. However, repetition of the experiments over longer periods of time (tables 4 and 5) shows clearly that these genes continue to decrease in frequency. Since the results in the populations parallel closely the predictions based on the mating experiments, it seems that, in these populations, the most decisive selective force is mating success. The viability of all four mutant types is good (Merrell, 1949a), but even so, yellow and cut were eliminated rapidly. The selection coefficient between alleles is, in many theoretical discussions, often assumed to be 0.001 or 0.01, but it should be pointed out that, for *y* and *ct*, the selection coeffi-

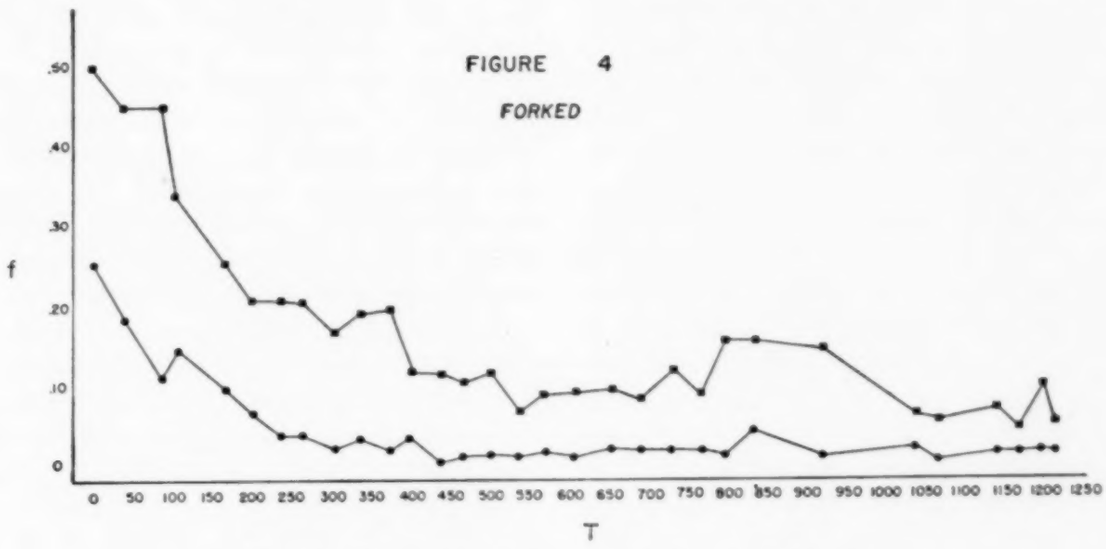

FIG. 4. Competition between forked and wild type

TABLE 5. *Competition in populations with forked and wild type alleles*

Generation or count	Day	Number of populations	N	% Males	q_m (obs.)	q_f^2 (obs.)
0	0	9	135	66.7	.500	—
1	39	9	1599	48.6	.450	.182
2	85	9	219	38.4	.452	.111
3	102	9	808	49.6	.337	.147
4	168	9	956	42.9	.251	.097
5	200	9	794	45.7	.204	.063
6	234	9	880	42.4	.206	.032
7	263	9	862	39.6	.202	.033
8	302	8	429	43.1	.168	.020
9	334	8	614	35.5	.188	.030
10	374	8	444	49.8	.194	.018
11	400	8	501	40.9	.117	.030
12	433	8	324	40.7	.113	—
13	465	8	431	40.8	.102	.008
14	500	8	413	48.5	.115	.009
15	533	8	328	51.6	.065	.006
16	564	8	817	51.2	.088	.010
17	607	8	401	49.6	.090	.006
18	651	8	442	47.5	.095	.017
19	687	8	317	47.0	.080	.012
20	727	8	266	47.0	.120	.014
21	764	8	384	42.7	.085	.013
22	798	8	273	46.9	.156	.007
23	830	6	455	48.6	.154	.038
24	921	5	330	51.5	.147	.006
25	1037	5	329	48.3	.057	.018
26	1068	5	318	60.4	.052	—
27	1136	5	486	48.6	.068	.008
28	1162	4	266	50.8	.044	.008
29	1196	4	222	60.8	.096	.011
30	1209	3	619	46.2	.049	.009

cient must be of the order of 0.9 even in the absence of any major viability effects.

In order to compare the observed and the calculated results it was necessary to put them on approximately the same time scale. The counts were usually made about once a month; the unit of time for the calculations was a generation. A generation in the population bottles was obviously less than thirty days because, in the populations, the mutant males were much less frequent than the wild type at the first count although equality was expected if they were the F_1 males, sons of heterozygous females. Differences in viability seem not great enough to produce this decrease. Moreover, with populations involving two or more mutations, Ludwin sometimes found crossover type F_2 females at the first count. The length of a generation, therefore, must have been somewhat more than fourteen days and less than thirty.

The length of generation is rather difficult to estimate in a population. It depends, not on the average length of life but on a factor more difficult to measure, the average span from the mid-point in the reproductive life of one generation to the mid-point in the reproductive life of the next. In a population these spans so overlap one another as to be nearly inseparable, and knowledge as to the percentage of progeny surviving to the reproductive period is not easy to obtain. However, an indirect estimate of generation length can be attempted from the yellow data. Yellow was chosen because male mating success was drastically lowered, but no other effect of the yellow gene was observed. The rapid decrease in the frequency of yellow seems to be attributable almost entirely to selective mating. If it is assumed that the effect on mating is the only effect of yellow in the populations, then the observed and the calculated curves should coincide. When the calculated curve is superimposed on the observed curve for the decrease in frequency of yellow, a rough estimate of the length of a generation in the population bottles can be obtained. The two curves are very similar in shape, which supports the validity of the assumption and simplifies the matching process. This procedure indicates that the calculated decrease in the frequency of yellow due to six generations of selective mating was reached after 144 days in the population bottles. The length of a generation, therefore, may be estimated to be about 24 days. This value is used for the rest of the populations.

If the mating system here postulated is actually operative in the population bottles, it should be detectable by an analysis of the frequency of the mutant gene in the males and females. If mating is at random, the frequency of the gene will appear to be about the same in both sexes. If the mutant males fail to mate as often

TABLE 6. *Comparison of gene frequency estimates in males and females*

Count	Yellow			Cut		
	q_f	q_m	$q_m - q_f$	q_f	q_m	$q_m - q_f$
0	.500	.500		.500	.500	
1	.148	.333	+.185	.084	.321	+.237
2	.170	.227	+.057	.055	.132	+.077
3	.089	.134	+.045	.045	.088	+.043
4	—	.077	+.077	—	.013	+.013
5	—	.028	+.028	—	—	.000
6	—	.006	+.006	—	.003	+.003
		$\overline{q_m - q_f}$	= +.066		$\overline{q_m - q_f}$	= +.062

Count	Raspberry			Forked		
	q_f	q_m	$q_m - q_f$	q_f	q_m	$q_m - q_f$
0	.500	.500		.500	.500	
1	.315	.443	+.128	.427	.450	+.023
2	.280	.350	+.070	.334	.452	+.118
3	.245	.260	+.015	.384	.337	−.047
4	.230	.282	+.052	.312	.251	−.061
5	.179	.241	+.062	.252	.204	−.048
6	.173	.178	+.005	.179	.206	+.027
7	.078	.134	+.056	.182	.202	+.020
8	.078	.061	−.017	.142	.168	+.026
9	—	.063	+.063	.173	.188	+.015
10	.078	.015	−.063	.134	.194	+.060
11	—	.084	+.084	.173	.117	−.056
12	—	.051	+.051	—	.113	+.113
13	—	.024	+.024	.089	.102	+.013
14	—	.033	+.033	.095	.115	+.020
15	—	.009	+.009	.078	.065	−.013
16	—	.013	+.013	.100	.088	−.012
17	—	.005	+.005	.078	.090	+.012
18	—	.011	+.011	.131	.095	−.036
19	—	—	.000	.110	.080	−.030
20	—	.014	+.014	.118	.120	+.002
21	—	.016	+.016	.114	.085	−.029
22	—	.017	+.017	.084	.156	+.072
23	—	—	.000	.195	.154	−.041
24	—	.044	+.044	.078	.147	+.069
25	—	—	.000	.134	.057	−.077
26	—	—	.000	—	.052	+.052
27	—	—	.000	.089	.068	−.021
28	—	.023	+.023	.089	.044	−.045
29	—	.063	+.063	.105	.096	−.009
30	—	.024	+.024			
31	—	—	.000			
32	—	—	.000			
33	—	.048	+.048			
		$\overline{q_m - q_f}$	= +.026		$\overline{q_m - q_f}$	= +.004

as the wild type, there will be fewer homozygous recessive females present than expected with random mating, and the frequency of the mutation will appear to be lower in the females than in the males. For a sex-linked gene, the frequency of the mutant in the males is obtained from the formula $p_m + q_m = 1$; in the females, from $p_f^2 + 2p_fq_f + q_f^2 = 1$. (The symbol p_m stands for the proportion of males with the dominant allele; q_m, for the proportion of males with the recessive: p_f^2 is the proportion of homozygous dominant females, $2p_fq_f$ the proportion of heterozygous females, and q_f^2 the proportion of homozygous recessive females, with p_f and q_f being the frequency of the dominant and recessive alleles in the females.) These equations assume random mating. If mating is random, q_m will approximate $\sqrt{q_f^2}$. If mating is not at random, the mutant males being less successful, q_m will be greater than the calculated q_f. Therefore, table 6 is most important in that it shows, for yellow and cut, values of q_m always larger than those of q_f. The raspberry populations show 24 values of q_m larger than q_f, but only 2 which are smaller. With forked, which had no mating effect, q_m was greater than q_f in 15 cases and smaller in 14, indicating that the differences between the estimates are essentially random in this case. These results are entirely in accord with the expectations based on mating performance. The average values of $q_m - q_f$ furnish another indication of the departure from random differences. These values are + 0.004 for forked, + 0.026 for *ras*, + 0.062 for cut, and + 0.066 for yellow, again emphasizing the consistent excess of q_m over the calculated q_f for *y*, *ct*, and *ras*.

The possibility also exists that q_f might be less than q_m because of the superiority of the heterozygous females over both homozygous types. This alternative explanation, however, is rather improbable. In the first place, if it were true, the mutant genes would not be eliminated from the populations, but would reach an equilibrium value. The evidence is quite clear that when the mutation is present in the populations, males carrying it are viable enough to survive in the population bottles. If a significant proportion of the

wild type females were heterozygous, their mutant male sons would appear in the populations. Hence, the disappearance of the mutant type from the populations indicates that the gene has been lost entirely, and no equilibrium exists. Furthermore, the results with forked, which shows no difference between q_m and q_f, yet persists longest in the populations, cannot be explained at all on this basis. Therefore, the explanation which best fits the facts is that selective mating is the major factor in the decrease in frequency of yellow, cut, and raspberry, and that forked persists longer because it does not impair mating efficiency.

Various evidence shows that selective factors other than non-random mating were also operative. The sex ratio in the populations usually showed an excess of females. In these populations, the females are evidently more viable than the males. Moreover, the data on forked in table 5 show a gradual decrease in its frequency even though it had no detectable effect on mating success. As a check for other effects of forked which might affect its selective value, longevity and egg production were compared for forked and its wild type allele. The longevity studies were made on 102 homozygous wild type adults and 120 forked flies with equal numbers of both sexes. The flies were transferred to fresh food every six days. The results are plotted in figure 5. The oldest individuals of both types lived to about the same age, but the median age at death was 29 days for forked and 44 days for the wild type.

For the egg production experiments, Lausanne Special wild type females were held for seven days in half pint bottles with Lausanne Special males, and then isolated in shell vials for 24 hours, after which the eggs in the vials were counted. Forked females were also placed with Lausanne Special males and treated in the same manner as the wild type females. The average production of 45 wild type females under these conditions was 39.09 $\pm$ 1.38 eggs; that of 44 forked females was 31.15 $\pm$ 1.69 eggs. The difference in egg production is statistically highly significant with a probability less than 0.01 of being due to chance since it is 3.67 times the standard error of the difference between means.

Therefore, the wild type has a selective advantage over forked in both the additional traits tested. Other differences could probably also be found. The decrease in the frequency of forked can thus

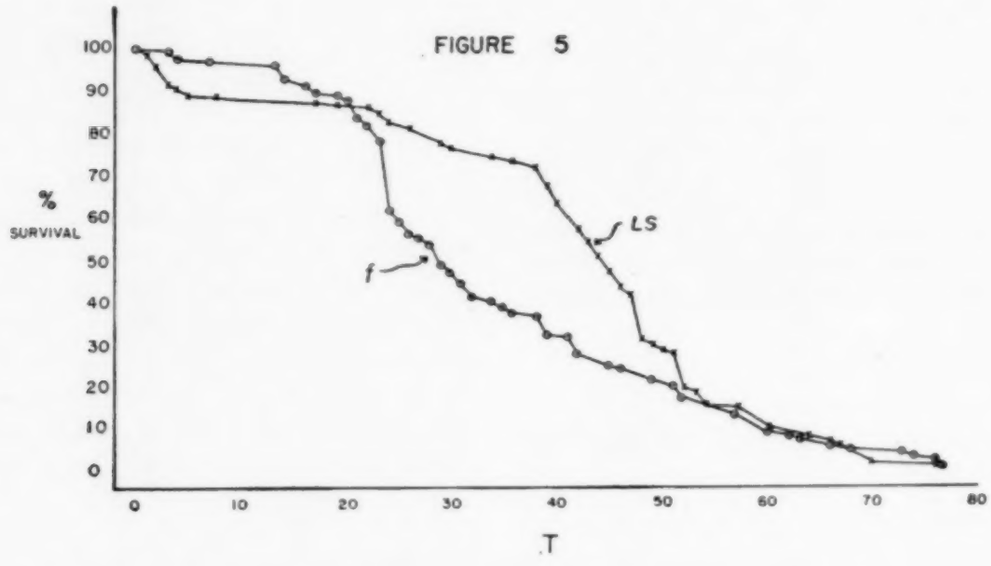

FIG. 5. Comparison of the survival of forked (f) and wild type Lausanne Special (LS) flies.

be accounted for as due to its selective disadvantage in traits other than mating.

Discussion

The results show quite strikingly how rapidly gene frequencies may change because of selective mating. The mating process appears to be a major selective mechanism in laboratory populations of Drosophila. Since the basic law in population genetics, the Hardy-Weinberg formula, assumes random mating, it is clear that this assumption must be tested before the equation can be used.

Selective mating may be of similar importance in wild populations, but its effect may be more difficult to detect. Most of the variations in natural populations are minor compared to the mutants used in these experiments so that selection would probably be less drastic. However, differences in mating behavior between two wild type strains of *Drosophila melanogaster* (Merrell, 1949b) indicate the possibility of selective mating even when phenotypically wild type individuals are involved.

As has often been pointed out, the true measure of success in an evolutionary sense lies in reproduction rather than survival. Therefore, among the survivors of the selective forces at work during developmental stages, selection may operate with undiminished or even increased effectiveness at the time of mating. While losses during development due to lack of food, predation, and so on may be primarily random, the courtship and mating between a male and a female of the same species can hardly be considered so. There is, ordinarily, an orderly sequence of events leading up to mating. The sequence may be considered as selection in a positive sense since not just the elimination of the less fit is involved. The necessity for a series of "normal" actions and reactions during courtship insures that mating will occur primarily between "typical" members of the species. Lack of response or inadequate response may interrupt courtship completely. Therefore, in higher animals, evolution may be determined not only by superior viability, fecundity, and similar factors, but also by the interactions between individuals of the same species. The factors governing these interactions are as yet relatively little known or understood, and their significance hardly investigated. The relative importance of the selection pressure exerted through the mating process compared to other selective forces is a question still largely unexplored. Darwin laid considerable emphasis upon the role of sexual selection in evolution, but subsequent authors for the most part have tended to minimize its role. Nevertheless, selective mating is undoubtedly of some importance in organisms with fairly well developed nervous systems.

Selective mating in which one type is favored over the other is unlikely to lead to sexual isolation between the alternative types, and is therefore not apt to play a part in making secure and permanent the differences between diverging populations during the process of speciation. However, within an evolving species, selective mating may act as a conservative force because the more extreme variants in a population are less apt to mate successfully. The existing gene frequencies will then tend to persist. It is even conceivable that new and otherwise better adapted types could fail to become established due to their inability to secure mates.

Summary

On the basis of mating experiments, the changes in the frequency of four sex-linked recessive mutants in populations of *Drosophila melanogaster* were predicted. The actual changes in gene frequency were then followed in laboratory populations. Each mutant was in competition with its wild type allele. The experimental results agreed closely with the predicted values, indicating that selective mating was a major factor in the decline in frequency of the mutant genes.

Literature Cited

Ludwin, I. 1951. Natural selection in *D. melanogaster* under laboratory conditions. Evolution, **5**: 231–242.

Merrell, D. J. 1949a. Selective mating in *D. melanogaster*. Genetics, **34**: 370–389.

——. 1949b. Mating between two strains of *D. melanogaster*. Evolution, **3**: 266–268.

——. 1950. Measurement of sexual isolation and selective mating. Evolution, **4**: 326–331.

Reed, S. C., and E. W. Reed. 1948. Natural selection in laboratory populations of Drosophila. Evolution, **2**: 176–186.

——. 1950. Natural selection in laboratory populations of Drosophila. II. Competition between a white-eye gene and its wild type allele. Evolution, **4**: 34–42.

Spencer, W. P. 1947. Mutations in wild populations of Drosophila. Advances in Genetics, Vol. I: 359–402. Academic Press, New York.

COMPARATIVE GROWTH BETWEEN AND WITHIN CLIMATIC RACES OF *ACHILLEA* UNDER CONTROLLED CONDITIONS

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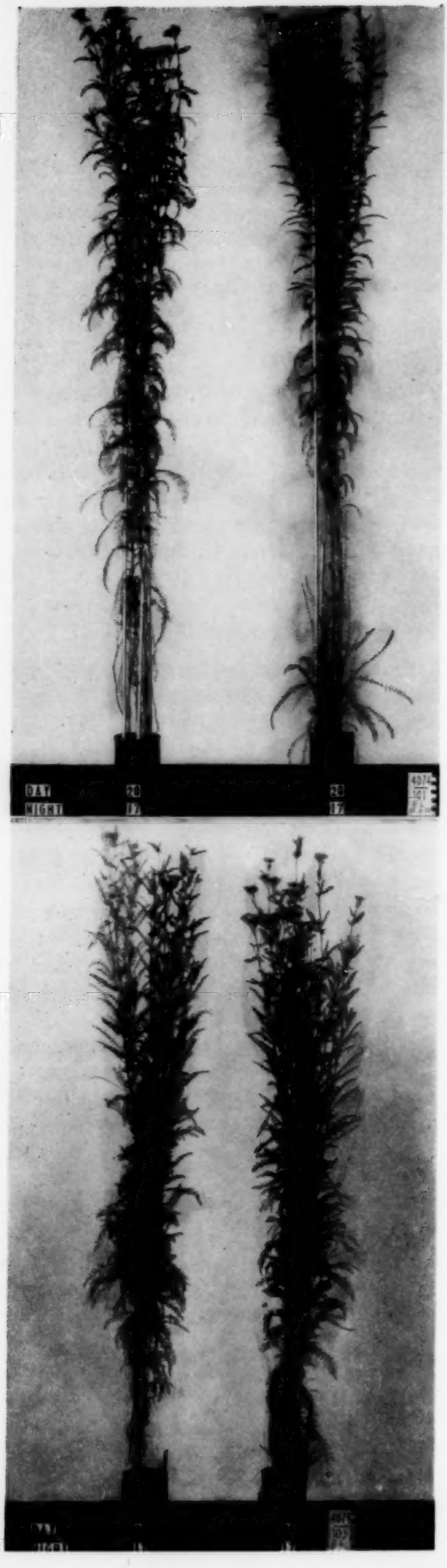
Since the classic investigations of Turesson (1922, 1925) the fundamental role of ecological races in the evolution of species of plants having a wide-spread distribution has been demonstrated by different investigators in divergent genera and families. The *Achillea millefolium* complex of the Compositae is a particularly revealing group to study from this point of view because it contains a large array of climatic races that are distributed in a wide range of environments in North America. In an extensive investigation of this group, Clausen, Keck, and Hiesey (1948) have analyzed a series of races from different altitudes along a transect in California ranging from sea-level to 11,000 feet, and from different latitudes extending from central California to Alaska and Swedish Lapland. This study consisted primarily of comparisons between populations of diverse races when grown in the same uniform garden, and their patterns of response and survival when transplanted as vegetatively propagated clones to gardens situated at three contrasting altitudes.

Few studies have as yet been made on the comparative growth of ecologic races in controlled environments. In the work cited above, preliminary work in this direction was reported on climatic races of *Achillea* that were grown during 1944 at different combinations of controlled temperatures and lengths of day in air-conditioned greenhouses of the California Institute of Technology at Pasadena. These controlled greenhouses have been described by Went (1943). The completion of the new Earhart Laboratory at Pasadena in 1949 with its greatly expanded facilities for controlling the external environment of growing plants has since made possible much more extensive studies (Went, 1950). The present report describes a more detailed study of the growth of a small sample of individuals of three selected races of *Achillea* under controlled temperatures in the Earhart Laboratory during 1950.

BACKGROUND DATA

Figure 1 shows duplicate clones of two individuals of a race of *Achillea borealis* originally from the San Joaquin Valley of California grown under controlled temperatures at the Earhart Laboratory at Pasadena. Each pair was subjected to the same environment for a period of 119 days. During this period the two divisions of each individual developed from small vegetative propagules to the mature flowering stages illustrated. The striking similarity in appearance, in growth rate, and in timing of development of vegetative and flowering states of each pair throughout the period of the experiment provides a demonstration of the reproducibility of growth and timing of development when clones having identical genetic composition are grown under the same conditions.

The curves of figure 2, on the other hand, reflect the diverse growth patterns that one can observe when parts of one individual are subjected to different controllable combinations of temperature and light. These curves show the rates of growth of flowering stems under different treatments of parts of the same plant illustrated in the upper half of figure 1. These hitherto unpublished data were ob-



tained during 1944 at the California Institute of Technology, and the experimental details are described by Clausen, Keck, and Hiesey (1948, p. 87–90). The contrasting responses of a single individual shown by the curves are obviously the result of physiological processes conditioned by the environment on the same hereditary material.

The sets of curves in figures 3 and 4, likewise obtained from the data of 1944, show how the growth rates of flowering stems of plants belonging to other climatic races of *Achillea* were affected by exposure to the same set of controlled environments as those indicated in figure 2. If one compares the curve-patterns for the three races shown in figures 2, 3, and 4 it is clear that the responses differ. For example, the beaded line in figure 2 reveals that the interior valley race developed flowering stems rapidly when grown at the continuous warm day and night temperature of 26° C., whereas the coastal race, figure 3, remained vegetative under the same conditions and developed no stems. Likewise, the changed relationship between the two curves shown by heavy solid lines in figures 2, 3, and 4 show that the relative growth rates of the three clones when subjected to two different night temperatures, 7° as compared with 17° C., are shifted markedly. On the other hand, there may be a remarkably parallel rate of development of the three races under the same environments, as, for example, when the three contrasting races are exposed to a day temperature of 17° C., a night temperature of 13° C., and

FIG. 1. Similarity of growth of clones at the same controlled temperatures.

Above: Two propagules of the individual 4074–101 of a race of *Achillea borealis* originally from near Selma, Fresno County, California, at 300 feet elevation, grown at a day temperature of 20° C. and a night temperature of 17° C.

Below: Two propagules of another individual of the same race, 4074–103, grown at a day temperature of 30° C. and a night temperature of 17° C. The small black and white scale beside the plant number is 1 dm. high.

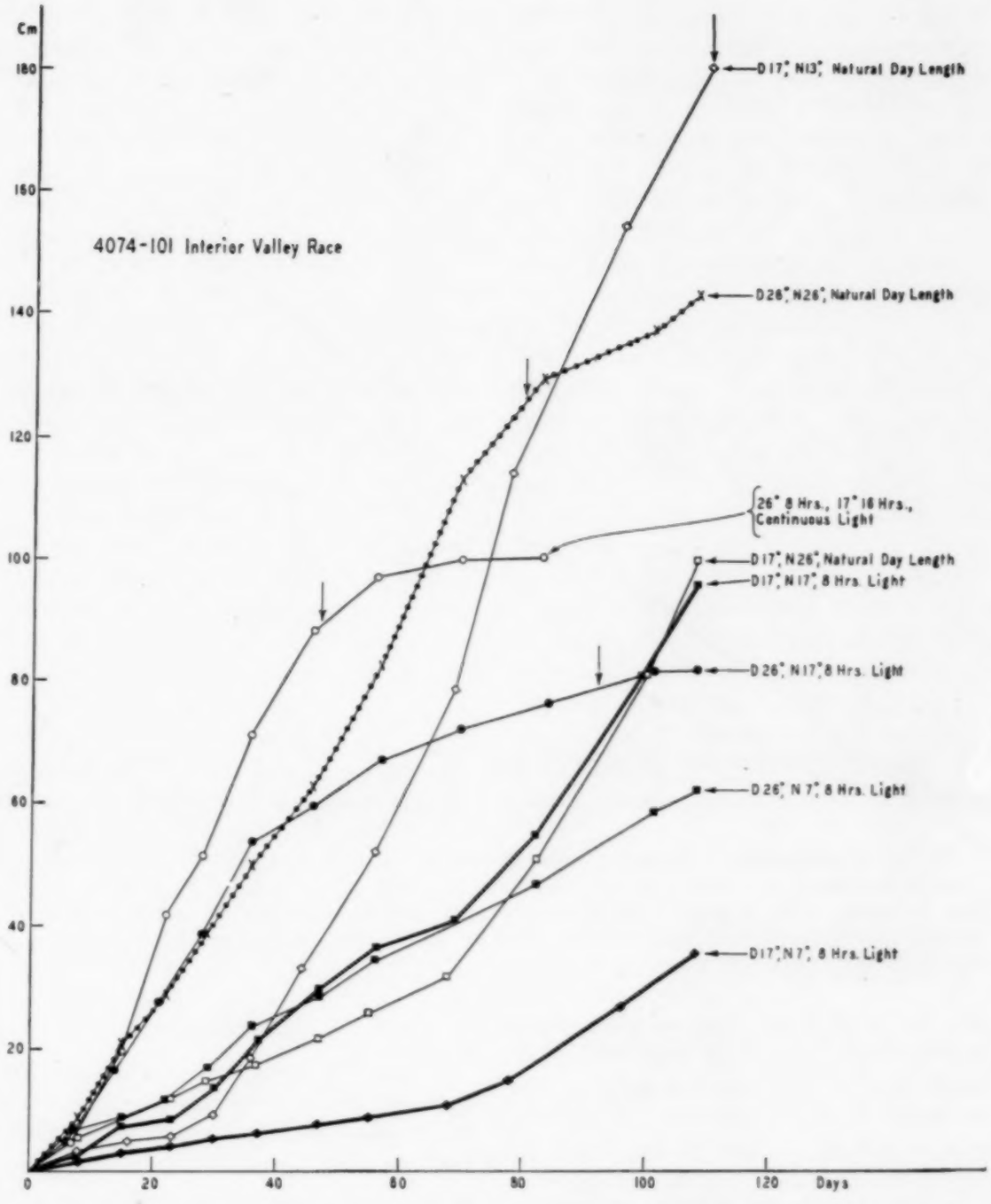


FIG. 2. Growth rates of flowering stems of propagules of clone 4074–101 from near Selma in the San Joaquin Valley, California (illustrated at the top of figure 1) when subjected to different combinations of controlled temperatures and periods of illumination. Heights of stems (vertical axis) are plotted against days (horizontal axis) of exposure to the environmental combination described for each curve. Arrows above curves point to dates of first opening of flowers; absence of an arrow indicates that the plant failed to develop flowers during the experimental period. D = day temperature, N = night temperature (in degrees Centigrade).

the natural day-length occurring at Pasadena in spring.

The growth curves obtained in the exploratory work of 1944 for single individuals representing contrasting races of *Achillea* made it possible to select conditions under which significant contrasts in growth response between given races

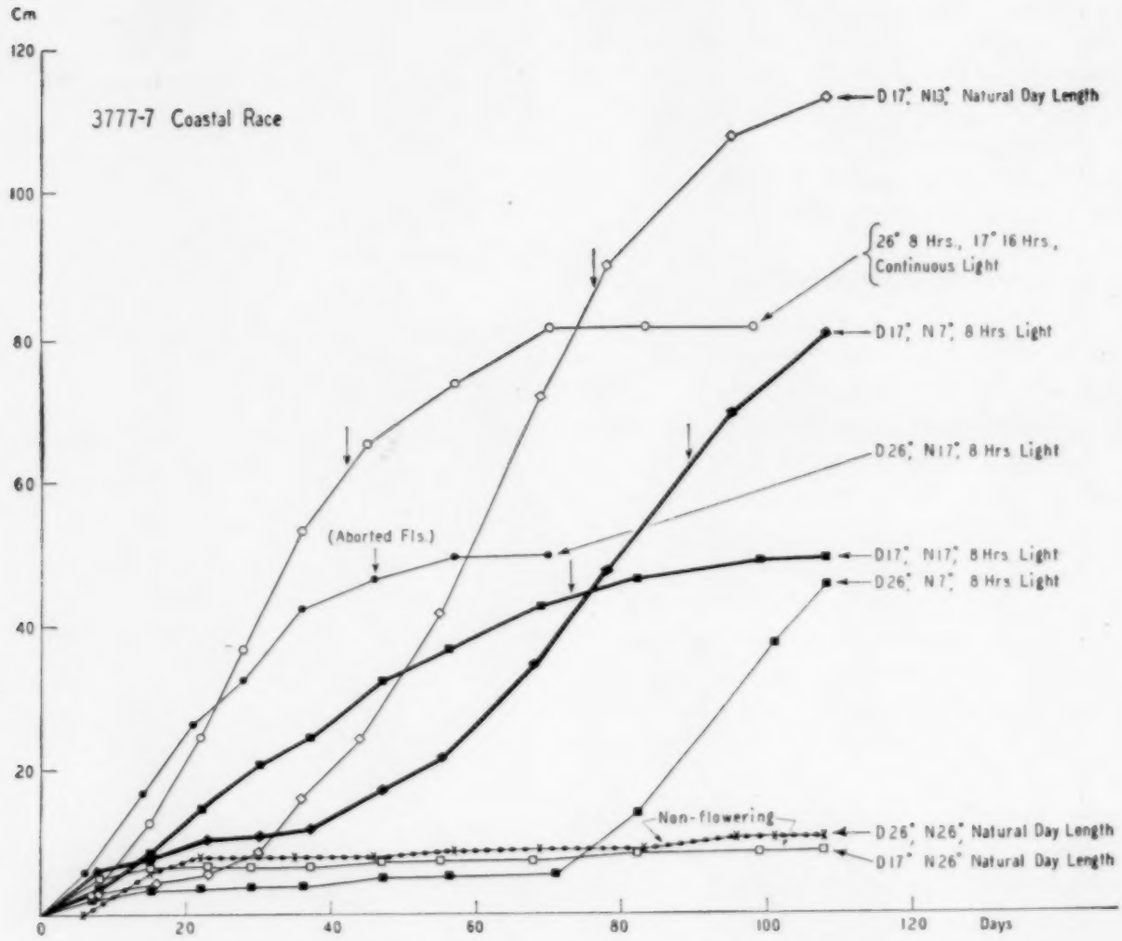


FIG. 3. Growth rates of flowering stems of propagules of a clone, *3777–7*, of a tall coastal race of *Achillea borealis* native near San Gregorio, San Mateo County, California, at 160 feet elevation. The propagules of this clone were grown under the combinations of temperature and periods of illumination described for each curve. See figure 2 for explanation of arrows and abbreviations.

might be predicted. One of the weaknesses of the 1944 data was that each climatic race of the fourteen studied was represented in the experiments by only a single individual. Since it has been repeatedly demonstrated that natural populations of *Achillea* from all kinds of habitats normally consist of an array of genetically distinguishable individual variants, it was desirable to compare the responses under controlled temperatures of individuals of a sample containing typical variants of the same natural population. This was accomplished in the 1950 experiments at the Earhart Laboratory which were limited to the study of three climatic races in four combinations of controlled temperatures.

MATERIALS

Population samples of six individuals each of three contrasting climatic races of *Achillea* were propagated vegetatively into parts as similar to each other as possible. The three races used were as follows:

1. *The maritime race.* The six individuals of the maritime race selected for study came from a population of *Achillea borealis* ssp. *arenicola* (Heller) Keck native to the cool, wind-swept coast of Sonoma County, California, near Bodega, close to sea-level. This population consists of relatively dwarf plants that in their natural environment with a year-long growing season always have active green leaves.

Another sample of this same population was used by Clausen, Keck, and Hiesey (1948) in their transplant studies, and details regarding the morphological characteristics, individual variability, and responses of this population when transplanted to different altitudes may be found in their publication. Typical of the species, this race is hexaploid with $2n = 54$ chromosomes. Studies during 1944 of a cloned individual of this population grown under controlled conditions indicated growth responses closely paralleling those of the plant *3777–7* shown in figure 3 belonging to a taller-stemmed coastal race originally from a habitat less exposed to extreme sea winds.

2. *Interior valley race.* The plants used in the present experiments were from a population of *Achillea borealis* from near Selma, examples of which are shown in figure 1. This race is native to the hot interior San Joaquin Valley of California. It is in active growth most of the year and is the tallest form known of the species. In morphology it contrasts markedly with the maritime race described above, and has been named as a separate species (*A. gigantea* Poll.), but it has the same chromosome number and is genetically fully interfertile with the maritime race (Clausen, Hiesey, and Nobs, 1951).

3. *High montane race.* The sample of six individuals of the high montane race came from a population of *Achillea lanulosa* ssp. *typica* Keck native to the upper Sierra Nevada of California at White Wolf, Yosemite National Park, at 8700 feet elevation. This race is tetraploid with $2n = 36$ chromosomes, like other forms of the species (Lawrence, 1946), and is one of a series of altitudinal climatic races of this species that occur on the western slope of the Sierra Nevada. In its native habitat it is subjected to long, cold winters during which it is covered with deep snow through a prolonged dormant period. It is somewhat comparable with the Aspen Valley race described by Clausen, Keck, and Hiesey (1948) but comes from a higher altitudinal zone. When grown in the mild coastal climate

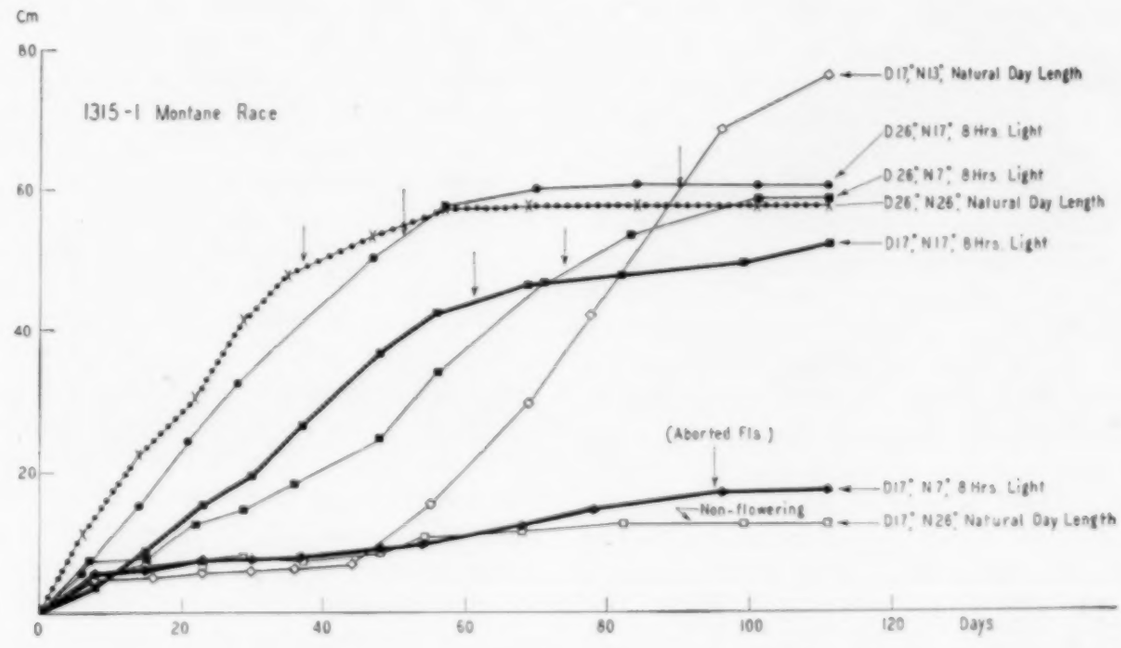


FIG. 4. Growth rates of flowering stems of one cloned individual, 1315–1, of a race of *Achillea lanulosa* originally from a meadow at 4600 feet altitude in the central Sierra Nevada of California at Mather, Tuolumne County. The propagules of this clone were grown under the combinations of temperatures and periods of illumination described for each curve. See figure 2 for explanation of arrows and abbreviations.

at Stanford it retains its characteristic of becoming winter-dormant, whereas the interior valley and maritime forms remain active all year in that environment.

The three races were originally collected from the wild as seeds and were grown to maturity in spaced plantings in the Stanford garden for a period of years during which comparative observations were made on their growth and annual periodicity together with numerous other populations likewise belonging to the *Achillea millefolium* complex. The plants of the three populations selected for the present study represent extremes of individual variation within each so far as could be determined by garden observations.

Methods

The plants used for the Earhart experiments were propagated as vegetative divisions of rootstocks at Stanford in the fall of 1949 and grown in soil in 5-inch flower pots. By early December the clones were well established. The soil was then washed from the roots and the plants were wrapped in paraffined paper and brought from Stanford to Pasadena by car. Before being brought into the Earhart Laboratory the plants were fumigated with methyl bromide to kill insects. The fresh weight of each propagule was then recorded and the plants were potted in plastic cups using sterilized river sand as a growing medium. They were watered with Hoagland's nutrient solution twice daily. In the Earhart Laboratory the Achilleas were kept for two weeks in quarantine in an isolated room maintained at 18° C. for checking against the possible survival of living insects. Afterwards they were all kept for another period of 14 days in a room maintained at 23° C. from 8:00 A.M. to 4:00 P.M. and at 14° C. from 4:00 P.M. to 8:00 A.M. The treatment of the plants was essentially the same as that described for concurrent experiments being conducted on the growth of races and interspecific hybrids of *Poa* (Hiesey, 1953).

Beginning January 16, 1950, the propagules of the three races of *Achillea* were subjected to four different temperature combinations, one under each condition, as follows:

1. Day temperature 20° C., night temperature 6° C.
2. Day temperature 30° C., night temperature 6° C.
3. Day temperature 20° C., night temperature 17° C.
4. Day temperature 30° C., night temperature 17° C.

In several instances where more than four propagules of a clone were available, two divisions were placed in the same condition in order to check the reproducibility of the responses, as illustrated in figure 1.

From 8:00 A.M. to 4:00 P.M. the plants were kept at one temperature and under natural sunlight passing through the water-cooled glass roof of the Earhart Laboratory, the intensity of which was approximately one-half of that out-of-doors. At 4:00 P.M. the plants were moved to the room providing the desired night temperature and kept there until 8:00 A.M. the next morning. During the four hours between 4:00 P.M. and 8:00 P.M. they were illuminated from an overhead bank of fluorescent lights interspersed with incandescent lamps having an intensity at the level of the average plant of approximately 500 foot-candles. At 8:00 P.M. these lights were turned off by an automatic time clock and the plants remained in the dark until 8:00 A.M. the next morning. All the plants, therefore, received 12 hours of light daily, namely, 8 hours of natural sunlight at the relatively high day temperature, and 4 hours of artificial light at the lower night temperature. In the present discussion the temperature of the period from 8:00 A.M. to 4:00 P.M. is referred to as "day temperature," and that from 4:00 P.M. to 8:00 A.M. as "night temperature," although the first four hours of the latter period were under artificial light.

The plants were closely observed during the experiments. Relative growth, development of flowering stems, size of leaves, health and vigor were recorded at two-week intervals. A photographic record was taken immediately before the experimental treatments were begun, approximately two months later, and again

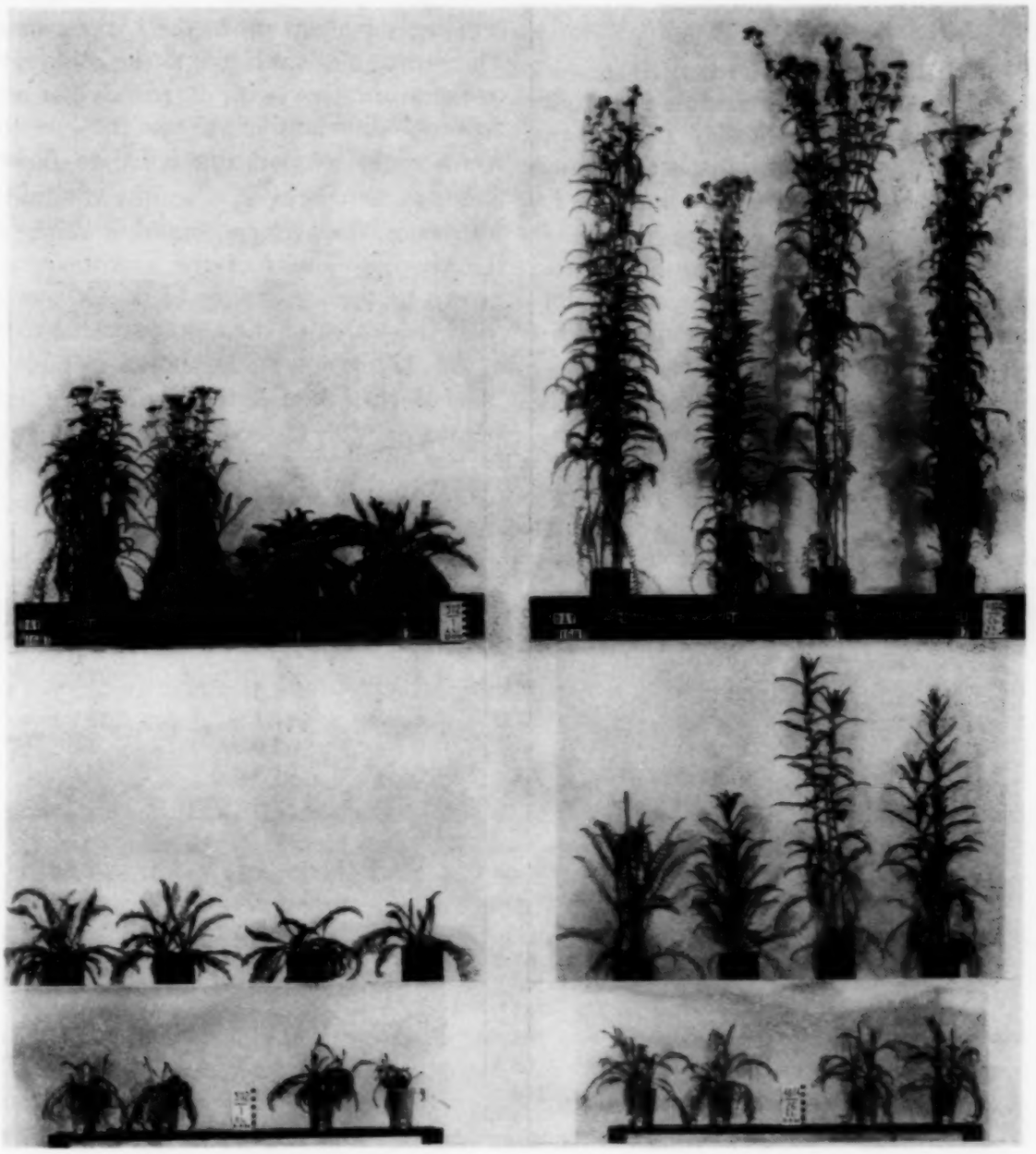


FIG. 5. Responses of a clone of a maritime race of *Achillea borealis* (left) as compared with those of a race from the San Joaquin Valley (right) when grown at four different combinations of controlled temperatures.

Left half: *Lowermost row*, four propagules of plant 5112–1 from Bodega, Sonoma County, California, on January 7, 1950, just prior to exposure to different temperatures; ***middle row***, the same propagules 63 days after treatment; *top row*, the same on June 6, 1950, 150 days after treatment. Day and night temperatures are indicated in degrees Centigrade.

Right half: Four propagules of plant 4074–26 from near Selma, Fresno County, California, treated the same as the maritime race. The topmost row, however, was photographed on May 3, 116 days after treatment, when in full flower.

All photographs reduced to the same scale; the scale beside the label is 10 cm. long. See table I for dry weights.

at the close of the experimental period after approximately five months. When harvested, both fresh and dry weights were determined on tops and underground portions.

Results

Typical differences in growth response between a clone of the maritime race and another of the interior valley race when subjected to the four combinations of controlled temperatures are shown in figure 5.

The four propagules of the maritime plant 5112–1 at the left of the figure are shown in three successive stages of growth. During the first two months relatively little difference in response was evident although the two propagules which were exposed to the cold night temperature of 6° C. gradually assumed a somewhat more vigorous appearance than the propagules subjected to the warmer night temperature of 17° C. Later the differences in response of the clones became increasingly evident up to the harvest date. The propagules subjected to the cold night temperature grew with extreme vigor and flowered abundantly, whereas those at the warm night temperature failed to flower although remaining in a healthy condition. Moreover, the average total dry weight of the six individuals of the maritime race grown at the cool night of 6° C. was at least twice that of their propagules grown at the 17° C. night, as shown in table I. The effect of the two contrasting day temperatures, 20° C. and 30° C., was rela-

Table I. *Total dry weights of cloned individuals of* Achillea *grown at four temperatures* (Grams)

Race and clone number	Nights 6° C.		Nights 17° C.	
	Days 20°	Days 30°	Days 20°	Days 30°
Maritime race:				
3776–1	22.0	25.0	4.5	12.5
3776–10	38.0	24.0	17.5	13.0
3776–23	26.0	38.5	2.0	5.5
3776–31	46.5	63.5	24.5	34.0
5112–1	60.0	49.5	26.5	31.5
5112–2	20.5	8.0	4.0	7.5
Means	35.50±6.38	34.75±8.14	13.00±4.51	17.33±5.02
Interior valley race:				
4074–14	46.0	63.5	36.5	52.0
4074–18	51.0	90.5	70.5	80.5
4074–26	112.0	87.0	66.5	60.0
4074–101	113.0	105.5	113.5	105.0
4074–102	67.5	68.5	—	29.5
4074–103	117.5	104.5	120.0	91.5
Means	84.50±13.60	86.58±7.16	81.40±15.60	69.75±11.36
High montane race:				
5105–1	24.0	27.5	9.0	16.0
5105–2	27.5	36.0	18.5	6.0
5105–3	21.5	11.5	10.5	17.5
5105–4	12.0	21.5	19.5	6.0
5105–5	17.5	16.0	9.0	—
5105–6	18.5	11.0	11.0	11.5
Means	20.16±2.21	20.59±4.01	12.91±1.95	11.40±3.13

TABLE II. *Heights of longest stems of cloned individuals of* Achillea *grown at four temperatures* (Centimeters)

	Nights 6° C.		Nights 17° C.	
	Days 20° C.	Days 30° C.	Days 20° C.	Days 30° C.
Maritime race:				
3776–1	19*	26	0	0
3776–10	41	46	0	0
3776–23	15*	66	0	0
3776–31	39	38	10*	0
5112–1	55	53	14*	0
5112–2	13*	19*	0	0
Means	45.0±5.01	45.8±6.75	—	—
Interior valley race:				
4074–14	110	91	185*	177
4074–18	137	114	179	162
4074–26	139	126	162	144
4074–101	148	130	185	179
4074–102	137*	100*	—	87*
4074–103	130	124	167	162
Means	132.8±6.38	117.0±7.01	173.3±5.30	164.8±8.16
High montane race:				
5105–1	41*	57	0	0
5105–2	62	63	0	0
5105–3	43	0	0	0
5105–4	35	21	0	0
5105–5	0	0	0	—
5105–6	4*	49	0	0
Means	46.6±8.01	47.5±9.28	—	—

* Stems immature at harvest date; heights not included in computing means. 0 = no stems produced.

tively small as compared with the effect of the differences in night temperature.

By contrast, plants of the San Joaquin Valley form, as exemplified by the clone 4074–26 in the right half of figure 5, grew generally better at the warm night temperature of 17° C. The four initial propagules, which were similar to each other at the beginning of the experiment, revealed visible differences in growth after 63 days (middle horizontal row). The two divisions subjected to a night temperature of 17° C. made faster growth than the two grown at the cooler night of 6° C. These differences continued to the conclusion of the experiment, 116 days after the start of the temperature treatments, as shown in the upper horizontal row of figure 5. Although the flowering stems which developed at the warm night temperatures were typically taller than those produced at the colder nights, the differences in dry weight between clone-members grown at warm and cold nights were not statistically significant, as shown in table I. At the colder nights the stems and leaves became thicker with more dry matter per unit area. There was a definite response in the interior valley race to differences in day temperature, growth being better at the 20° C. day than at 30° C. in the clone illustrated. The responses to day temperatures varied widely, however, among individuals of the same population. The

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FIG. 6

TABLE III. *Number of days elapsing between first appearance of flowering stems and first anthesis*

	Nights 6° C.		Nights 17° C.	
	Days 20° C.	Days 30° C.	Days 20° C.	Days 30° C.
Maritime race:				
3776–1	—	55	—	—
3776–10	59	33	—	—
3776–23	—	61	—	—
3776–31	69	57	—	—
5112–1	58	35	—	—
5112–2	—	—	—	—
Means	62.0±3.51	48.2±5.88	—	—
Interior valley race:				
4074–14	95	59	—	86
4074–18	78	54	55	79
4074–26	66	57	61	60
4074–101	82	62	56	75
4074–102	—	—	—	—
4074–103	81	61	64	62
Means	80.4±4.64	58.6±4.54	59.0±2.12	72.4±4.97
High montane race:				
5105–1	50	23	—	—
5105–2	37	28	—	—
5105–3	28	—	—	—
5105–4	47	20	—	—
5105–5	—	—	—	—
5105–6	—	28	—	—
Means	40.5±5.02	24.7±1.97	—	—

significance of intra-population variation will be discussed in later paragraphs.

The non-flowering of the maritime race observed at the warm night temperature of 17° C. may in part be due to a prolonged delay rather than a complete inhibition. The propagule of plant 5112–1 illustrated in figure 5, for example, which was grown at the day temperature of 20° C. and at a night temperature of 17° C., developed a few flower primordia towards the end of the experimental period. No evidence of flower bud development was found, however, in any of the propagules of this race exposed to the 30° C. day and the same warm night. The conclusion is clear that the warm night temperature of 17° C. definitely curtails flowering on the maritime race, but not on the interior valley race.

The growth responses of the high Sierran race of *A. borealis* from White Wolf to the four combinations of temperature were in many respects similar to those of the maritime race, especially with regard to the stimulation both of total growth and of flowering when exposed to cold nights of 6° C. as compared with the warm nights of 17° C. This is illustrated by figure 6 which shows sets of clones of three different individuals of the White

FIG. 6. Individual differences in the responses of three clones of the high montane race of *Achillea lanulosa* to four combinations of temperature. The propagules were photographed 163 days after their first exposure to the day and night temperature combinations indicated in degrees Centigrade.

FIG. 7

TABLE IV. *Ratio, tops : roots, of cloned individuals of* Achillea *grown at four temperatures*
(Based on mean dry weights of six plants of each race)

	Nights 6° C.		Nights 17° C.	
	Days 20° C.	Days 30° C.	Days 20° C.	Days 30° C.
Maritime race	1.57±0.67	1.78±0.23	1.29±0.23	1.56±0.11
Interior valley race	2.04±0.14	1.55±0.20	3.19±0.86	2.23±0.38
High montane race	0.82±0.03	1.21±0.13	0.74±0.14	0.79±0.11

Wolf race at the end of the experimental treatments. Table I showing total dry weights reveals the fairly consistent improvement in total growth at the lower night temperature for the individuals of this race.

Although there was a resemblance in dry weight yield and flowering response between the maritime race of *A. borealis* and the high montane race of *A. lanulosa,* the two races differed markedly in their relative rates of development. In its native environment the maritime race is winter-active, whereas the high montane is winter-dormant until late in spring. In the controlled environments the high Sierran plants remained in slow vegetative growth of rosette leaves at all four temperatures until late April, then quickly developed flowering stems on the divisions that were exposed to the cold night of 6° C. The time required for the appearance of first flowers after the first initiation of visible stem development in the maritime, interior valley, and high-Sierran Achilleas is shown in table III. Such differences in rate of development are doubtless of ecological significance in climates having growing seasons of very different length. The interior valley race initiated flowering stems early in January and was the first to reach full flowering, the exact date depending upon the particular temperature combination; in the maritime race stems were not started until mid-March and flowering in the earliest plants was therefore six weeks to two months later than in the valley race; and in the high montane race the initiation of stem development was delayed until May, but afterwards developed very rapidly. Within all three races individual variation in rate of flowering development was marked, but the differences between the races overshadowed the intra-population variation.

Another characteristic of the high-montane race is the greater development of roots and underground rhizomes in relation to top growth as compared with the maritime and interior valley races. This is reflected in table IV showing the mean ratios of tops to roots computed from dry weights. The low value of this ratio for the high montane race reflects the relatively greater development of underground parts which are doubtless of importance in the storage of food reserves in this naturally winter-dormant form. This contrasts markedly with the interior valley race which has extensive top growth, especially when grown at the day temperature of 20° C. in combination with warm nights of 17° C. where the mean value of the ratio is 3.19 ± 0.86 as compared with 0.74 ± 0.14 for the high montane race. The wide variations in this ratio for the

FIG. 7. Individual variation in response to controlled temperatures among three clones of the same maritime population of *Achillea borealis.*

Uppermost row: Four propagules of the plant 3776–1 originally from Bodega, Sonoma County, California, grown at the different day and night temperatures indicated. Figures denote degrees Centigrade.

Center row: A corresponding set of propagules of the plant 3776–10.

Lowermost row: A similar set from the plant 3776–23.

All photographs were taken on June 6, 1950, 150 days after the indicated temperature treatments were started; all are reproduced at the same scale. The height of the scale beside the label is 10 cm.

interior valley plants under the different combinations of temperature reflect the high modifiability of this character in the same clones, but the differences between the lowland and high montane races are nevertheless so great that they are undoubtedly significant.

Individual Variation Within the Same Populations

From the viewpoint of natural selection and evolution one of the most basic questions is the extent of physiological variation among individuals of a given natural population as compared with the physiological differences between different races. In numerous garden cultures of many populations of *Achillea* from diverse natural habitats grown at Stanford it has been observed that natural populations of *Achillea* of the *millefolium* complex are universally genetically variable.

Examples of differences in response among individuals of the three races to the controlled temperatures is illustrated in figure 6 showing propagules of three different clones of the high Sierran race at the conclusion of the Earhart experiments, figure 7 showing a corresponding stage of three clones of the maritime race, and figure 8 four clones of the interior valley race.

Individuals of all three races differ among themselves in their response to the cool day temperature of 20° C. as compared with the warm day of 30° C. In the maritime race, for example, the plant 3776–23, illustrated in the bottom row of figure 7, was favored in growth by a high day temperature of 30° C. more than by the cooler day of 20° C. regardless of whether the clone was grown at a cold night of 6° C. or a warm one of 17° C. A tendency for just the opposite response was observed in the clone 3776–10 illustrated in the middle row of the same figure. The third clone, 3776–1, shown in the top row, was intermediate in this respect. It is of interest that in the earlier transplant experiments of Clausen, Keck, and Hiesey the clone 3776–23 proved to be considerably more successful when transplanted to the relative severe mid-altitude climate at Mather than either clone 3776–10 or 3776–1, all three of which, however, are equally successful when grown at Stanford. Although it is not possible at this stage of study to relate the patterns of response under controlled temperatures at the Earhart Laboratory to physiological characteristics that may be of importance for successful growth and survival in different climates, such observations are suggestive. A clone of *A. lanulosa* native to the middle altitudes at Mather and highly successful there (1315–1, figure 3) showed responses under controlled conditions rather similar to those observed for the maritime plant 3776–23. The active growing season at the mid-Sierran station is characterized by extremes in diurnal temperature with warm days and cold nights, and plants native to this area are particularly successful under these conditions.

Individual differences in growth and development at the controlled temperatures are likewise clearly evident in the interior valley race, as figure 8 shows. Plant 4074–14, shown at the upper left of the figure, developed flowers more rapidly when exposed to cold nights of 6° C. than at warm nights of 17° C., whereas the reverse was true of the clone 4074–18 shown at the upper right. Another plant, 4074–102 (not illustrated), deviated even more widely from the others of this same race in being extremely late in flowering under three conditions tried (cf. table II); the data are incomplete for 4074–102 due to the accidental death of one of the clone-members in the early stages of the experiments.

The high-montane race from White Wolf displayed individual variation not only in rate of development of flowering stems in the clones exposed to cold night temperatures, as illustrated in figure 6, but also in such characteristics as production of total dry matter, shown in table I,

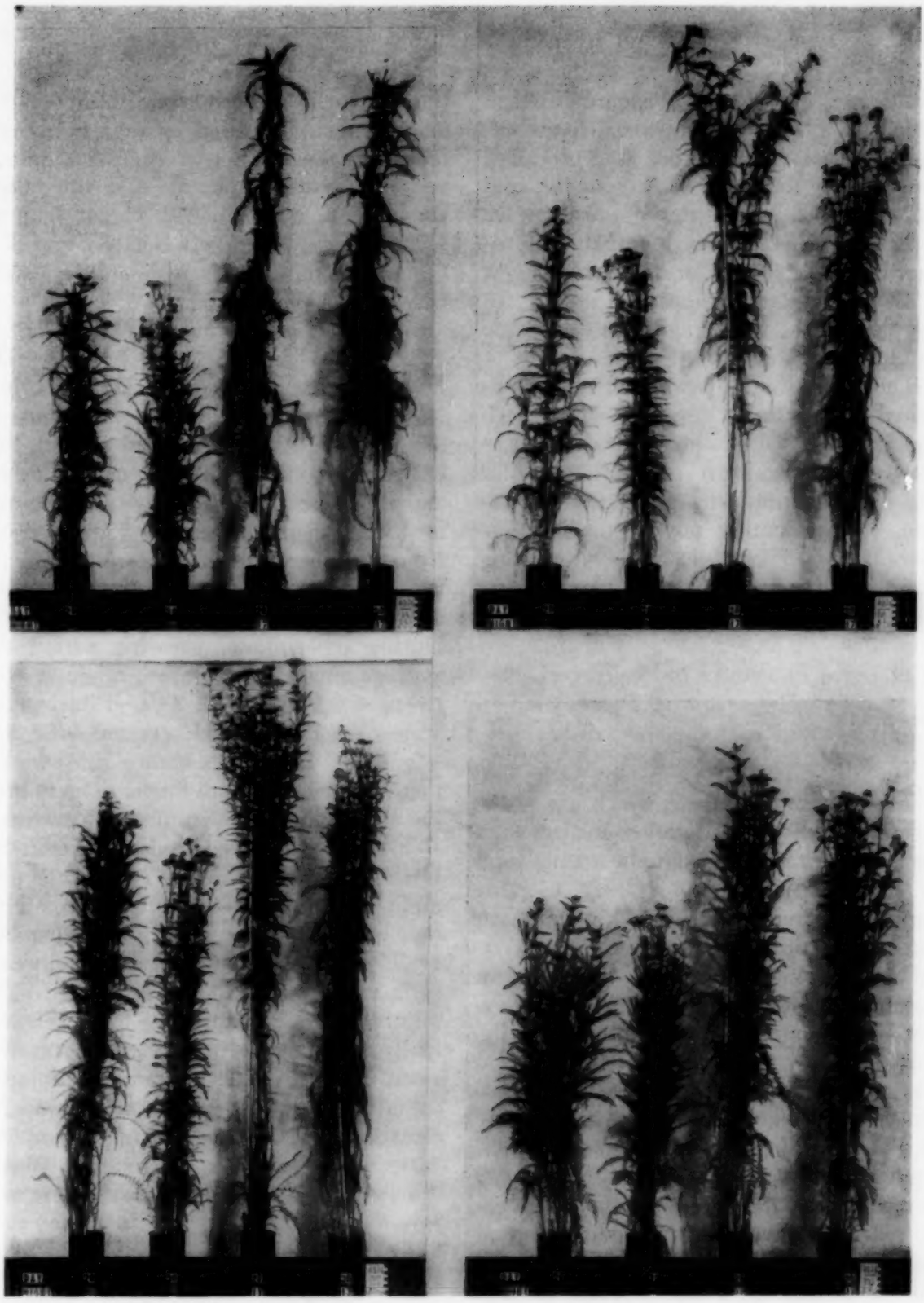

FIG. 8. Individual variation in response to controlled temperature combinations among four clones of the same San Joaquin Valley population of *Achillea borealis*.

Upper left: Four propagules of the plant 4074–14 originally from near Selma, Fresno County, California, grown at different day and night temperatures. Temperature treatments, from left to right, as follows: Day 20° C., night 6° C.; day 30° C., night 6° C.; day 20° C., night 17° C.; and day 30° C., night 17° C.

Upper right: The same experiment on propagules of the plant 4074–18.

Lower left and lower right: The same experiment on propagules of the plants 4074–101 and 4074–103, respectively.

All photographs were taken on May 3, 1950, 116 days after the temperature treatments were started; all are reduced to the same scale. The height of the scale beside the label is 10 cm.

and in height of flowering stems, listed in table II. The individual 5105–1, for example (upper row, figure 6), produced considerably more dry matter at the cold nights of 6° C. than at the warm nights of 17° C., whereas the clone 5105–3 produced more material at the warm night of 17° C. in combination with the 30° C. day than at the 6° C. night with the same day temperature (table I).

The conclusion is clear that while individual differences in response within each population are conspicuous, the differences between the races are of a higher order and overshadow intra-population variation. In evaluating physiological differences between ecologic races an understanding of intra-population variation is, however, essential.

Climatic Features of Native Habitats in Relation to Temperature Responses of the Races

The graphs in figure 9, which were constructed from U. S. Weather Bureau data and are reproduced from the monograph of Clausen, Keck, and Hiesey (1948), summarize temperature and precipitation data from three stations located in climates approximating those of the natural habitats of the maritime, interior valley, and high-montane races used in this study. All of these stations lie approximately in the same latitude (38° N.) and hence the length of day does not differ significantly in these. The data for the maritime climate at Point Reyes shown in figure 9 reveal the small amplitudes of both seasonal and diurnal variations in temperature as indicated by the closeness of the curves of average maximum and average minimum daily temperatures. The diurnal range throughout the year lies mostly between 8° and 15° C.

The temperatures for Fresno in the more interior but still lowland San Joaquin Valley show much greater amplitudes not only in the annual cycle from summer to winter, but also in the diurnal fluctuation shown by the difference between the average maximum and average minimum temperatures. The average maximum and minimum temperatures during midsummer are conspicuously higher than at Point Reyes, being between 18° and 37° C., and in midwinter considerably lower, between 3° and 14° C.

The montane climate at Huntington Lake at 2134 meters (7000 ft.) elevation in the Sierra Nevada of California has large seasonal and diurnal variations in temperature, like the Fresno station, but both the maxima and minima are lower, and a long period of freezing temperatures in winter distinguishes the high Sierran from both the Central Valley and the maritime climates. Accordingly, the active growing season is much reduced in length. During the summer growing period the average diurnal temperature range lies between + 9° and + 23° C. and during the winter dormant period between − 8° and + 6° C. In both summer and winter there are wide random fluctuations in both the maximum and minimum temperatures.

The success of the Bodega race in the Earhart Laboratory when exposed to cold night temperatures of 6° C. in combination with a day temperature of either 20° or 30° is consistent with the low temperatures prevailing in its native habitat. The failure of the maritime race to flower normally when exposed to warm night temperatures of 17° C. is of critical interest inasmuch as this night temperature is clearly above the range of night temperatures experienced in its native habitat. This race from a climate with generally low day temperatures tolerates, however, warmer days than are usual for this maritime climate, as evidenced by the healthy growth in the Earhart Laboratory at day temperatures of either 20° or 30° C. These day temperatures are well above the average maxima at Bodega but within the range of the extreme maxima at this locality.

The capacity of the San Joaquin Valley race to grow with success when exposed to

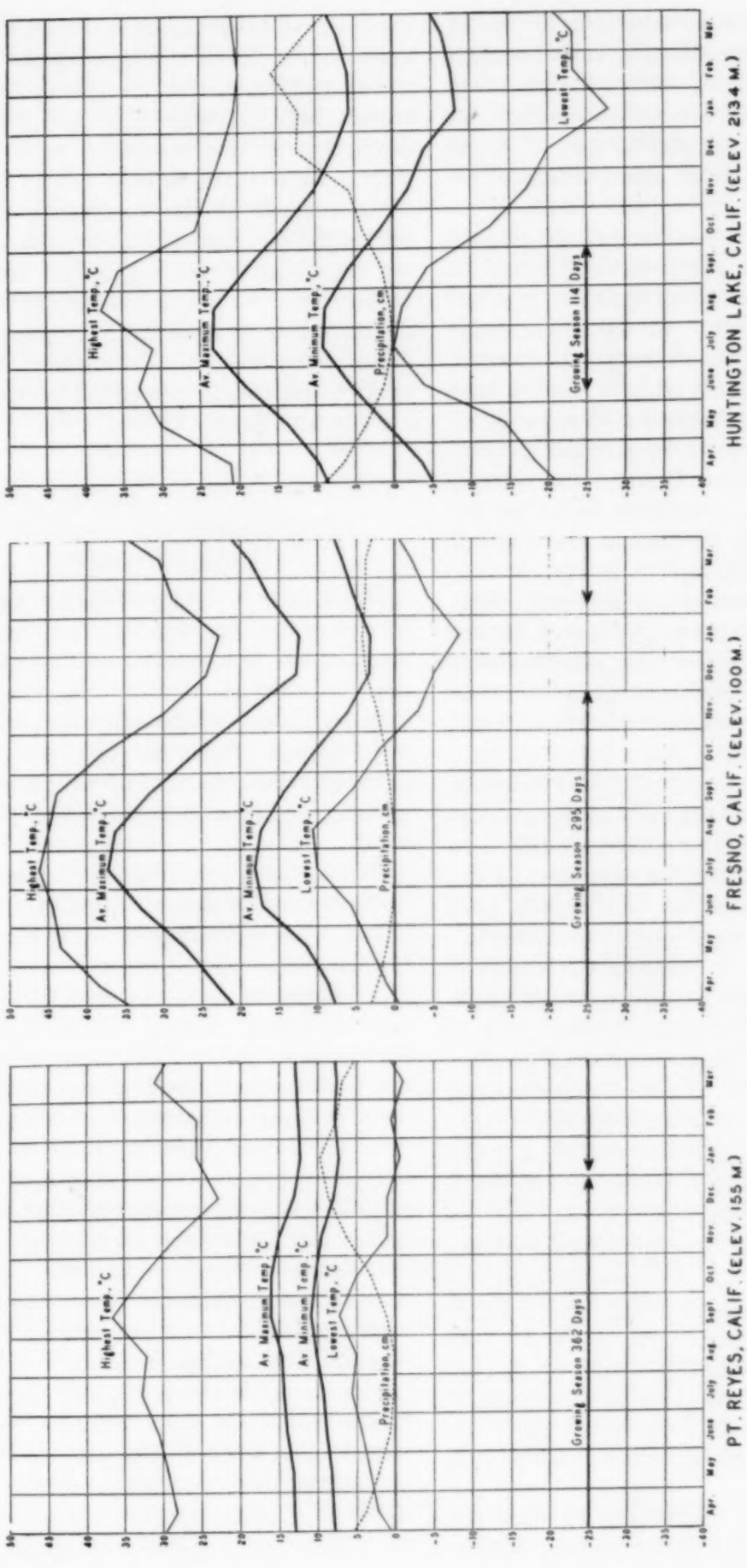
Highest Temp., °C
Av. Maximum Temp., °C
Av. Minimum Temp., °C
Lowest Temp., °C
Precipitation, cm
Growing Season 302 Days
PT. REYES, CALIF. (ELEV. 155 M.)
Growing Season 295 Days
FRESNO, CALIF. (ELEV. 100 M.)
Growing Season 114 Days
HUNTINGTON LAKE, CALIF. (ELEV 2134 M.)

FIG. 9. Seasonal curves of maximum and minimum temperatures, precipitation, and average growing season (frost-free period) of maritime, Central Valley, and Sierran high-montane localities in central California. Constructed from U. S. Weather Bureau data averaged for 39 years from the Pt. Reyes station (maritime), 42 years from Fresno (Central Valley) and 24 years from Huntington Lake (high montane).

the warm night temperature of 17° C. is likewise consistent with the summer temperature range in its native habitat. Its ability to grow almost as successfully at the colder night temperature of 6° C. is of equal interest since the average minimal temperature during the winter in its native habitat is approximately 5° C. and this race remains winter-active there. In view of the great amplitude of seasonal temperature variation in its native habitat and its all-year activity, it is noteworthy that the San Joaquin Valley race showed a greater tolerance to a range of night temperatures in the Earhart experiments than did the Bodega race. The valley race grew somewhat better at the cooler day of 20° C. than at the warmer one of 30° C. although it is clearly able to develop satisfactorily at the latter temperature. In its native habitat it is exposed to much higher day temperatures than its range of tolerance under the controlled temperatures would seem to indicate.

The Sierran race of *Achillea lanulosa* is subjected to a radically different climatic pattern so far as temperatures are concerned than either the maritime or the interior valley races of *A. borealis* just mentioned. During the short season when temperatures in the montane environment permit active growth, the average minimum temperatures range between 5° and 9° C., and the average maxima from 19° to around 24° C. It is of significance that in the Earhart Laboratory night temperatures of 7° C. favored normal growth and flowering of the high Sierran race, whereas night temperatures of 17° C. were definitely unfavorable to flowering. Day temperatures of 30° C. were apparently slightly more favorable for the rapid development of flowering stems of this race than day temperatures of 20° C. although the plants were clearly tolerant of both. The average summer maxima in its native environment fall within this range.

An important difference between diurnal temperatures in a natural out-of-doors environment as compared with those used in the Earhart experiments is that under natural conditions the diurnal extremes both for maximum and minimum temperatures are normally realized for only a short time, usually for an hour or less, whereas in the controlled environments the day and night temperature levels were maintained for 8 and 16 hours, respectively. The effects on growth and development of prolonged exposure to a given temperature as compared with a short exposure to the same temperature are not sufficiently known.

The Evolutionary Significance of Physiological Variability Within Populations

If we are to understand the interplay between external forces and biological mechanisms that govern natural selection and evolution in wild species, it is necessary to know intimately the inherited physiological characteristics of ecologic races and the manner in which they respond to known factors of the environment.

From the preliminary studies under controlled conditions at the California Institute of Technology it is evident that there are deep-seated inherited physiological differences between naturally occurring ecological races. These differences can be observed as growth responses, but the physiological mechanisms underlying them are still unknown. It is also clear from the present studies that even within each local population there are significant physiological differences between individuals. The individuals of a population express these differences in their responses both to environments in controlled experiments and to the fluctuations in climate in their natural habitat.

For example, in the maritime Bodega race it is evident from table II that individuals like 3776–1 and –31 are favored by warmer days, and that in contrast plant 3776–10 of the same population is favored by cooler days. Nevertheless, all the in-

dividuals of the Bodega population tolerate the range of climatic and edaphic variations prevailing at that locality. Moreover, they would probably be able to occupy and successfully compete in a great number of habitats in the outer coastal zone of central California, the ecological zone to which the Bodega locality belongs.

The climatic conditions at any one locality are constantly changing around certain norms. The norms are expressed in climatic graphs like those of figure 9, but the conditions at any one locality are in perpetual change, daily from hour to hour, seasonally throughout the year, periodically from one year to the next, and in time through geological epochs. Adaptiveness to the current fluctuating changes in the environment is partly effected by the range of tolerance of each individual or biotype and partly by the physiological variability within the local population augmented by the interchange of heredity within and between adjacent populations of the same ecological zone. The epochal changes in environment, on the other hand, are reflected in terms of evolutionary changes in the race or species.

All the individuals of a local population are subjected to the current fluctuating changes in the environment and respond to them according to their individual physiological make-up. They tolerate these changes, although certain of the individuals are favored by changes in one direction, and others in the opposite. In some periods certain kinds of biotype will be favored, in others different kinds. For the survival of the race or the species in constantly changing environments, it is therefore of great importance that the variability is preserved and that the genetic mechanisms controlling the inheritance permit the perpetuation of sufficiently diverse populations. Species that are composed of sufficiently diverse components, both on the level of the local population and on the level of the ecologic race, would be expected to become the survivors through epochs of competition. The local population is one of the basic elements in preserving potential variability. The ecological race is a category of higher order preserving an even greater degree of divergence, and the species itself is the overall composite expression of its diverse elements.

The results of the present investigation emphasize the importance of individual physiological variability as well as differences between ecological races as elements in the dynamics of natural populations. Further inquiry into the specific nature and origin of physiological differences within and between ecologic races must rest upon basic physiological studies of specific processes pursued on the background of an analysis of the genetic and ecological structure of different kinds of species.

The deductions made from studies on growth and development in experiments performed in gardens of contrasting climates and in controlled greenhouse experiments in the Earhart Laboratory make it clear that the plant as an individual is physiologically quite resilient, that a local population is even better equipped to cope with changes in the environment because of its greater physiological resources. On a higher level, the resiliency of the species as a whole to changes of geologic periods is greatly enhanced by the genetic and physiological potentialities preserved as reservoirs in its constituent ecologic races occupying a range of environments.

Acknowledgments

The writer is especially indebted to Dr. F. W. Went, Director of the Earhart Laboratory, for the opportunity to use its unique facilities, and to Mr. G. P. Keyes, superintendent of the Earhart Laboratory, for kind help throughout the experiments. The valuable suggestions of Dr. Jens Clausen in the preparation of this manuscript are deeply appreciated.

Summary

The growth of three contrasting ecologic races of *Achillea* belonging to the

millefolium complex and originating from a maritime, an interior valley, and a high montane habitat was studied under four combinations of controlled temperatures at the Earhart Laboratory of the California Institute of Technology at Pasadena. Each race was represented by a sample of six plants selected to represent typical intra-population variability. The plants were cloned vegetatively and the propagules grown in the controlled environments for a period of approximately five months. The plants of the maritime race, originating from the coast of central California, grew with vigor and flowered freely when exposed to cold night temperatures of 6° C. in combination with a day of either 20° C. or 30° C., but failed to flower or to grow satisfactorily at the warm night temperature of 17° C. A race originally from the San Joaquin Valley, on the other hand, thrived at both the cold and warm night temperatures, being favored by the latter. A race from the high Sierra Nevada originally from an altitude of 8700 feet resembled the coastal race with respect to growth and flowering at the different day and night temperatures, but differed in its rate and timing of development. Individual variation among the six plants of each race was clearly evident in their patterns of response, but the differences between the races overshadowed the individual variation within the races. The responses of the three races to the controlled temperatures are examined in relation to the characteristics of the climates of their original habitats. The significance of individual physiological variants within local natural populations in relation to the larger physiological differences between ecological races and to the evolution of the species is discussed.

Literature Cited

Clausen, Jens, David D. Keck, and William M. Hiesey. 1948. Experimental studies on the nature of species. III. Environmental responses of climatic races of *Achillea*. Carnegie Inst. Wash. Publ. No. 581. iii + 129 pp.

Clausen, Jens, William M. Hiesey, and Malcolm Nobs. 1951. Genetics of climatic races and species in *Achillea*. Carnegie Inst. Wash. Yearbook No. 50, pp. 115–117.

Hiesey, William M. 1953. Growth and development of species and hybrids of *Poa* under controlled temperatures. Amer. Jour. Bot. **40**: 205–221.

Lawrence, William E. 1947. Chromosome numbers in *Achillea* in relation to geographic distribution. Amer. Jour. Bot., **34**: 538–545.

Turesson, Göte. 1922. The genotypical response of the plant species to the habitat. Hereditas, **3**: 211–350.

——. 1925. The plant species in relation to habitat and climate. Hereditas, **6**: 147–236.

Went, F. W. 1943. Plant growth under controlled conditions. I. The air-conditioned greenhouses at the California Institute of Technology. Amer. Jour. Bot., **30**: 157–163.

——. 1950. The Earhart Plant Research Laboratory. Chronica Botanica, **12**: 93–107.

BIOSYSTEMATIC STUDIES IN *ASTER*. II. ISOLATING MECHANISMS AND SOME PHYLOGENETIC CONSIDERATIONS [1]

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INTRODUCTION

In a previous paper (Avers, 1953), a summary was given of crossing relationships among seven of the heterophyllous asters. Limited interfertility was indicated by the production of a small number of fertile F_1 hybrids at the diploid and tetraploid levels. Certain features relating to effective gene flow were mentioned briefly in connection with their bearing on species criteria. In the present paper, an attempt will be made to indicate a possible phylogenetic pattern in this group. Although the evidence is still rather meager, certain trends in the evolutionary pattern can be discerned and it is believed that this study may perhaps add to the general understanding of taxonomically critical groups which contain complexes of closely related heteroploid species.

GENERAL COMMENTS

On the basis of morphology, the series HETEROPHYLLI Nees appears to be a natural subdivision of the section EUASTER Gray. The heterophyllous asters comprise herbaceous perennials which are usually found in woodlands and along their edges. All the species are eglandular and have petiolate leaves. The heads are borne on paniculiform inflorescences except for *A. ciliolatus* Lindl., which has a more or less corymbiform inflorescence. Although the rays are usually blue to pale violet, white-rayed forms are occasionally found in most of these species.

The geographic distributions (fig. 1), compiled from information on herbarium specimens, show that these species, with the exception of *A. ciliolatus*, are more or less restricted to eastern North America. *Aster anomalus* Engelm. and *A. texanus* Burgess have rather restricted ranges and, interestingly, they are the only heterophyllous species known to be exclusively diploid. In general, most of the species are sympatric with at least one other heterophyllous aster and in many cases are sympatric with almost all closely related species.

Cytologically, the species form a polyploid complex with diploid ($n = 9$), tetraploid ($n = 18$), and octaploid ($n = 36$) taxa represented. At least three species contain both diploid and tetraploid races. The one octaploid species, *A. ciliolatus*, is boreal in distribution which suggests that its origin may be correlated with the disturbances subsequent to the Pleistocene glaciations.

THE DIPLOID SPECIES

There are at least five diploid taxa although the evidence for one of them, *A. shortii*, is based only on a single plant collected in northeastern Iowa. While several plants were used to determine the ploidy level extant in each species, the collections were made mostly in limited areas (Avers, 1953).

Aster anomalus Engelm. This species is endemic to the Ozarks and is usually found associated with limestone bluffs. Its distribution overlaps completely with

[1] This paper is part of a thesis submitted to the Graduate School of Indiana University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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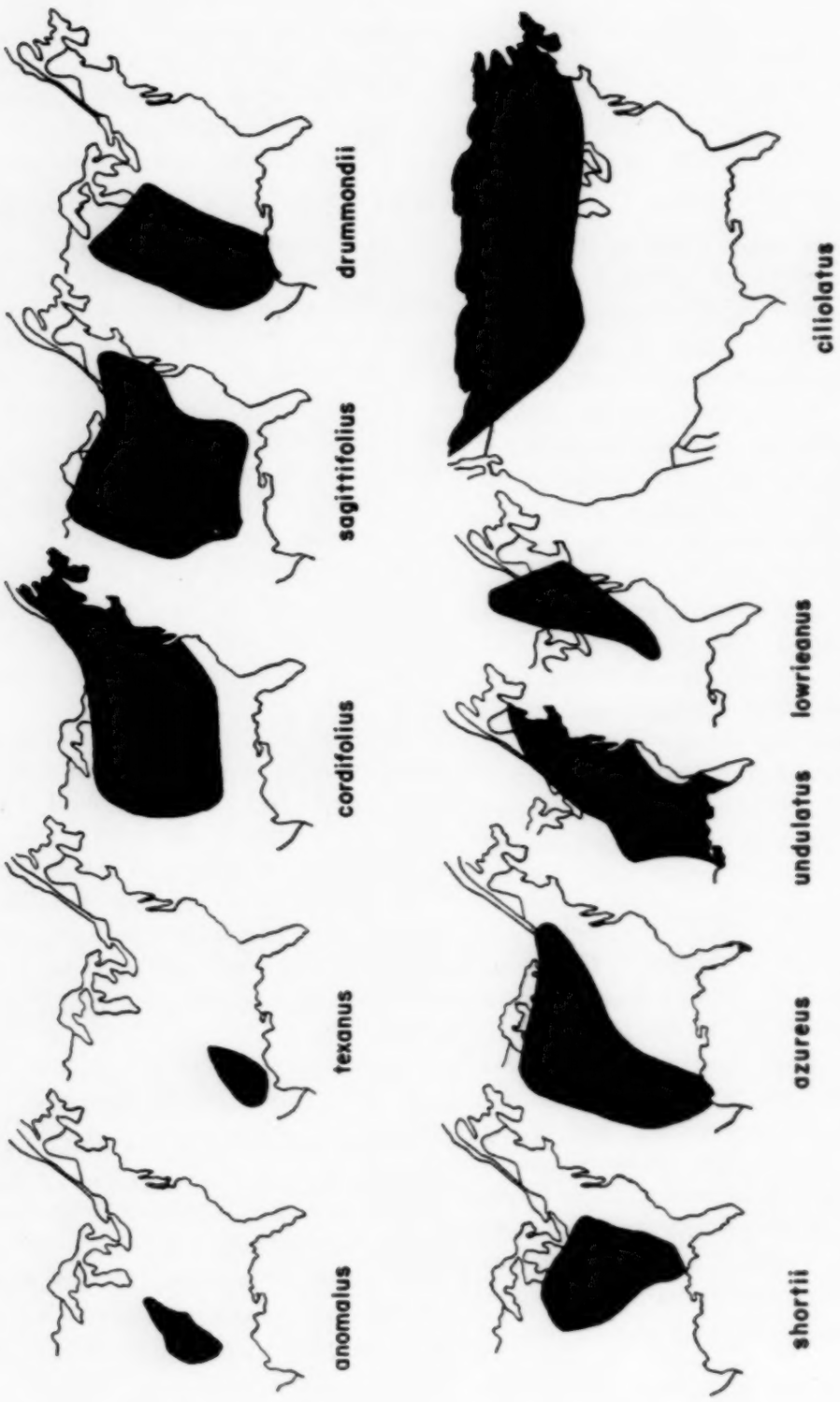

FIG. 1. Geographical distribution of the species of heterophyllous asters.

A. cordifolius and *A. sagittifolius* and partially overlaps with that of diploid *A. texanus*. The distribution of diploid *A. shortii* is not known, but *A. anomalus* and *A. shortii* do occur in the same region in Illinois.

Aster anomalus does not cross with *A. cordifolius* or *A. texanus* and actually stimulates selfing in the former species. According to Clausen (1951), these results indicate that *A. anomalus* may be a distinct comparium rather than a cenospecies. Although an occasional F_1 hybrid, which is pollen-fertile, is produced in crosses with *A. sagittifolius*, the first backcross generation consists of a few individuals which die in the seedling stage. The interaction of the recombined F_1 hybrid genome with that of the recurrent parent produces physiological disturbances which lead to the death of these later hybrid derivatives. Thus, there appears to be considerable genic divergence of the *A. anomalus* and *A. sagittifolius* complements although there is sufficient chromosomal homology to allow regular bivalent formation in the F_1 generation.

Cytological observations of pollen mother-cells of the artificial tetraploid F_1 *A. anomalus* ♀ × *shortii* ♂ showed a high frequency of multivalent formation which implies considerable chromosomal homology in these two species. These F_1 hybrids were 90 per cent pollen-fertile.

It may be that *A. anomalus* branched off early in the phylogeny of this group or, possibly, developed strict barriers to interspecific gene transfer more rapidly than did the other members of this group. The past distribution of *A. anomalus* is not known but today it occurs on glaciated as well as unglaciated terrain. Its presence on the former probably represents recent migration from a southern or Ozarkian center.

Aster texanus Burgess. This species is endemic to Texarkana and its distribution may represent the remains of a preglacial species, or recent divergence from one of the other diploid HETEROPHYLLI. Morphologically, *A. texanus* is closest to *A. drummondii*, a tetraploid species. Interspecific crosses between *A. texanus* and *A. cordifolius* or *A. sagittifolius* yield partially fertile F_1 progeny with a high degree of chromosome homology (Avers, 1953). It is possible that *A. texanus* represents an originally subspecific divergence from either *A. cordifolius* or *A. sagittifolius*, which developed more efficient reproductive isolation subsequent to geographic isolation. Although there appears to be some intergradation where these three species overlap in distribution, more intensive study is required to determine whether this phenomenon is truly intergradation, or whether it represents parallel evolutionary tendencies in certain morphological features. The characteristic lax branching habit of *A. texanus* occurs in other Texas asters (Shinners, 1950) and may also obtain in the heterophyllous asters in and near this region. Thus, lax branching in Arkansas specimens of various HETEROPHYLLI may merely represent parallel evolution rather than intergradation.

The partial sterility of the F_1 hybrids *A. texanus* × *cordifolius* and *A. texanus* × *sagittifolius* indicates genic divergence or chromosomal rearrangements which are manifested in discordant gene recombinations subsequent to meiosis in the hybrids. On this basis and the slight morphological distinctness, specific rank is retained for *A. texanus*.

Aster sagittifolius Wedem. The relationship of *A. sagittifolius* with *A. anomalus* and *A. texanus* has already been discussed (see above). The artificial F_1 hybrid *A. sagittifolius* × *cordifolius* is partially pollen-sterile (30 to 40 per cent) which indicates a certain degree of genetic divergence of these two species. This mechanism of restricting gene exchange is further enhanced by ecological isolation, and possibly, other factors. Herbarium studies have indicated that there is little intergradation of these two species in nature although they are sympatric. Cytological examination of meiosis in pollen mother-cells of F_1 *A. sagittifolius* × *cordi-*

folius and F_1 *A. sagittifolius* × *texanus* showed the presence of 2 to 4 unpaired chromosomes in about 25 per cent of the cells, which is correlated with the percentage sterility of the F_1 progeny. This chromosomal differentiation may be due to small structural changes such as deletions, duplications, and inversions. From the results of hybridization experiments and herbarium studies it is proposed that *A. sagittifolius* is a distinct taxonomic species which is capable of limited gene exchange with all the diploid representatives in this group.

Aster cordifolius L. This species has been studied through analyses of artificial hybridizations, natural populations, and herbarium specimens. It is ecologically distinct from the other heterophyllous asters in that it inhabits only those portions of the population site which are the most moist and heavily shaded. This ecological preference is apparently reflected in the thin, membranaceous texture of the leaves.

Aster cordifolius is specifically distinct from *A. anomalus* since hybridization between them is unsuccessful, and it is isolated from the other diploids by ecological preference and partial sterility of the F_1 hybrids.

Crosses of the diploids to the tetraploid species has been most successful with *A. cordifolius,* perhaps because of the more numerous attempts. Most of the information obtained from these crosses pertains to the tetraploid species and therefore they will be discussed below. In one cross, *A. cordifolius* ($n = 9$) × *A. shortii shortii* ($n = 18$), three triploids were obtained which were 90 to 95 per cent pollen-fertile and which morphologically approached the female parent species. Pollen mother-cell meiosis showed a range of chromosome configurations from 0 to 4 trivalents, 5 to 12 bivalents, and 1 to 9 univalents. This indicates at least partial chromosome homology among the *cordifolius* and two *shortii* genomes. By inference from the above cross, it would seem that diploid *A. shortii* and *A. cordifolius* also have partially homologous genomes.

The Tetraploid Species

Although chromosome counts were made of all species in this group, the two tetraploids *A. cordifolius* and *A. lowrieanus* were not available in time for crossing experiments.

Aster shortii shortii Lindl. Crosses with the tetraploids *A. sagittifolius, A. undulatus,* and *A. drummondii* resulted in seed sets of 13.4, 1.5, and 3.3 per cent, respectively, as compared to 46.5 per cent seed set in the intersubspecific crosses with *A. shortii* ssp. *azureus* (Avers, 1953). All the F_1 hybrids were pollen-fertile, but the F_2 and backcross generations of *A. shortii* × *A. sagittifolius* showed reduced fertility, and those of *A. shortii* × *A. drummondii* showed a marked decrease in vigor as well. The results of the artificial hybridizations indicate that *A. shortii* is reproductively isolated, at least partially, from the three tetraploids mentioned above.

Several factors indicate an autopolyploid origin for tetraploid *A. shortii* although this cannot be stated unequivocally until the natural species is compared with synthetic autotetraploids. The presence of a diploid race suggests autotetraploidy but some allopolyploids closely resemble one parent to the exclusion of the other (Howard and Manton, 1946; Manton, 1950), and have been mistaken for autopolyploids on a morphological basis. No multivalent associations have been observed in this species. The high frequency of trivalents and cells containing more than 9 bivalents in the triploid F_1 *A. cordifolius* × *shortii* indicates extensive duplication of chromosome material in the two *shortii* genomes. Further evidence of this sort is furnished by the occurrence of as many as 5 quadrivalents in the artificial tetraploid F_1 *A. anomalus* ♀ × *shortii* ♂. However, no multivalents were found in hybrids between *A. shortii* and other tetraploid heterophyllous asters.

Until the autotetraploid is synthesized it is assumed that *A. shortii shortii* is an

autotetraploid, or possibly a segmental allotetraploid.

Aster shortii azureus (Lindl.) Avers. That *A. shortii azureus* and *A. s. shortii* are co-specific was suggested by high percentage seed set in the initial interspecific crosses, as well as by the complete fertility of the F_1 generation. It is suggested that geographical isolation allowed divergence of *A. s. azureus* from *A. s. shortii* morphologically and ecologically, but that subsequent reproductive isolation has not occurred. These subspecies are largely allopatric but their distributions overlap in a limited area in the north central United States. Artificial F_1 hybrids between these two subspecies were morphologically similar to *A. s. azureus* in many important taxonomic characteristics so that it is impossible at present to ascertain the extent of intergradation in the region of geographical overlap.

Chromosome counts of material from the northern and southern range extremities all indicated the haploid chromosome number of 18. This species is mostly incompatible with the other tetraploid HETEROPHYLLI, or produces an occasional unthrifty F_1 hybrid in interspecific crosses (Avers, 1953).

Aster sagittifolius Wedem. Tetraploid populations of this species have been found only in southern Indiana, while the diploid appears to be more extensively distributed.

Crosses of *A. sagittifolius* with *A. shortii azureus* and *A. undulatus* failed but successful crosses were secured with *A. shortii shortii* and *A. drummondii*. The seed set in the initial interspecific crosses did not exceed 25 per cent, which may be indicative of some incompatibility mechanism operative early in the production of hybrid zygotes. The F_1 hybrids were pollen-fertile and no multivalents were observed in the pollen mother-cells.

Crosses between diploid *A. cordifolius* ♀ and tetraploid *A. sagittifolius* ♂ yielded one F_1 hybrid with a somatic chromosome number of 35. The hybrid presumably resulted from the union of an unreduced egg ($2n = 18$) with a reduced male gamete ($n = 18$), one of which lacked a chromosome. This hybrid was completely pollen-sterile and cytological studies of pollen mother-cells revealed the regular presence of 17 bivalents and 1 univalent. It is not known whether the pairing is auto- or allosyndetic, but in either case the high number of bivalents indicates duplication of chromosome material in the two *sagittifolius* genomes. The loss of a single chromosome may be the contributing factor to gametic sterility in this hybrid. It may be the same type of situation as found in monosomics of *Nicotiana tabacum* (Clausen and Cameron, 1944), i.e., differential viability depending upon which of the chromosomes is missing from the complement. This evidence of chromosome homology between the *sagittifolius* genomes may indicate either autopolyploidy or segmental allopolyploidy.

Taxonomic mergence of *A. sagittifolius* and *A. drummondii* (Shinners, 1941), or *A. sagittifolius* and another species would be premature since the mode of origin of tetraploid *A. sagittifolius* is in doubt at present.

Aster drummondii Lindl. Partial reproductive isolation of *A. drummondii* from *A. sagittifolius* and *A. shortii* ssp. *shortii* is accomplished by markedly reduced seed set in the initial interspecific crosses and by degeneration of later hybrid derivatives. Crosses between *A. drummondii* and *A. undulatus* were successful only with the former species as female parent. No evidence of intergradation in nature is available since these two species are now allopatric.

Crosses of *A. drummondii* with F_1 *A. sagittifolius* ♀ × *shortii* ♂ yielded triple hybrids which showed a marked decrease in vigor. Several of the hybrid seedlings were sub-lethal. These crosses demonstrate the possibility of gene transfer in allopatric species through the intermediary of a third species with which they are sympatric.

The F_1 hybrid *A. cordifolius* ♀ × *drummondii* ♂ ($2n = 36$) contained two iden-

tical *cordifolius* genomes and two *drummondii* genomes since this hybrid also arose presumably by the union of an unreduced egg and a reduced male gamete. Only a few pollen mother-cells were examined and these contained 15 to 17 bivalents. Whether or not the pairing is allosyndetic, the occurrence of a high proportion of chromosome pairs indicates considerable homology between the *drummondii* genomes. The occurrence of univalents suggests the possibility of some structural changes so that some *drummondii* chromosomes will not pair with one another or with *A. cordifolius* chromosomes. This may also imply segmental allopolyploidy.

Aster undulatus L. This species is the only representative of the HETEROPHYLLI on the Coastal Plain of the United States. Chromosome counts from populations in all extremities of the range indicated only the tetraploid condition (Avers, 1953). *Aster undulatus* was shown to be reproductively isolated from the other species in this group to a greater or lesser extent. There may be limited hybridization with asters in other EUASTER series since Fernald (1902) reported a population containing *A. novi-belgii* × *undulatus* in Massachusetts, and similar individuals have been seen by the author in herbarium studies of Georgia material. The extreme polymorphy of *A. undulatus* prompted Burgess (*in* Small, 1913) to split this species into many, but examination of his types showed them to be minor variants or hybrid derivatives. Introgression was not pronounced in Indiana in two natural populations containing *A. undulatus* and *A. shortii* ssp. *shortii*, and extensive intergradation does not appear to occur in nature.

Geographic and reproductive barriers separate this species from others in the group. The characteristic cordate-clasping petiole, unique in the HETEROPHYLLI, may have resulted during geographic isolation, or may be due to an initial hybridization with a *novi-belgii* type, at the diploid level, followed by chromosome doubling.

Aster cordifolius L. A single tetraploid specimen was obtained from central Pennsylvania which was morphologically indistinguishable from diploid members of this species. Pollen mother-cells contained 18 bivalents and, occasionally, 16 bivalents and a chain of 4. It cannot be said whether autopolyploidy is involved without extensive sampling of natural populations. It is possible that this specimen represents a sporadic tetraploid individual occasionally found in diploid populations. The presence of a chain of 4 chromosomes, however, may indicate structural hybridity as found by Müntzing (1941) in *Galeopsis tetrahit*.

Aster lowrieanus Porter. This species is mostly confined to the Appalachian region and closely resembles *A. cordifolius*, with which it is sympatric. Cronquist (oral communication) suggests that *A. lowrieanus* represents an allopolyploid derivative of *A. cordifolius* × *laevis* since it is somewhat intermediate between these two species morphologically. *Aster laevis* L. is apparently hexaploid (Darlington and Ammal, 1945; Delisle, unpub.) while *A. cordifolius* is diploid and possibly tetraploid. Although conventional allotetraploid origin involves chromosome doubling of a diploid interspecific hybrid, it is possible that a stable tetraploid species may result from a diploid × hexaploid combination. The tetraploid derivatives of such a cross would be reproductively isolated from the parent species because of the ploidy barrier.

If the genomic constitution of *A. laevis* is assumed to be *A A B B B′ B′* and that of *A. cordifolius* to be *A A* or *A′ A′*, then their progeny would be *A A B B′* or *A A′ B B′*. Homology between the *A*-genomes and between the *B*-genomes might result in regularity of meiotic pairing (18 bivalents), and fertility, if little structural hybridity was present among the chromosomes of the *A*- and *B*-genomes. Darlington (1930) found that the F_1 hybrids of *Prunus domestica* ($n =$

24) × *P. cerasifera* ($n = 8$) frequently showed configurations of 16 bivalents at meiosis. Ljungdahl (1924) crossed *Papaver nudicaule* ($n = 7$) with *P. nudicaule* var. *striatocarpum* ($n = 35$) and found that the F_1 hybrids were fertile and regularly showed 21 bivalents at metaphase I. Backcrosses of the F_1 hybrids ($n = 21$) to *P. nudicaule* ($n = 7$) yielded progeny with 14 bivalents at meiosis. However, in another cross, *P. nudicaule* ($n = 7$) × *P. radicatum* ($n = 35$), Ljungdahl reported little fertility of the F_1 hybrids despite regular disjunction of the 21 bivalents at anaphase I. Another example of this sort was reported by Collins *et al.* (1929) in a cross involving *Crepis biennis* ($n = 20$) and *C. setosa* ($n = 4$). The hybrid, *C. artificialis,* had 12 bivalents that regularly disjoined at meiosis, but later study showed that it was not true-breeding.

One example of this type has occurred in *Aster* in the origin of the F_1 hybrid *A. cordifolius* ♀ × *turbinellus* ♂. Diploid *A. cordifolius* ($n = 9$) was crossed with polyploid *A. turbinellus* Lindl. ($n =$ c. 50) and yielded two hybrids with the somatic chromosome number of 34. At meiosis there is regular disjunction of the 17 bivalents and, as a consequence, these hybrids are 90 to 95 per cent pollen-fertile. The pairing may be auto- and allosyndetic according to the following scheme:

($n = 9$) *cordifolius* × *turbinellus* ($n =$ c. 50)
9 *A* ↓ 9 *A*, 16 *B*
F_1 ($2n = 34$)
(9 *A* + 9 *A*) + (8 *B* + 8 *B*) = 17 bivalents

Although the original chromosome count of 48 to 50 pairs was found for *A. turbinellus,* a reduction in number is necessary to explain the chromosome constitution of the F_1 hybrids. It is possible that those heads used in making the crosses arose after somatic reduction (Menzel, 1950, 1952; Menzel and Brown, 1952), or the original count may have been made from a polyploid branch of a chimaeral plant.

Evidence of an $n = 8$ series in *Aster* is provided by Clausen, Keck, and Hiesey (1940) in studies of diploid and tetraploid *A. adscendens* Lindl. It is possible that *A. turbinellus* represents an allopolyploid derivative of $n = 8$ and $n = 9$ species.

The Octaploid Species

Aster ciliolatus Lindl. This species is geographically distinct from the other HETEROPHYLLI because of its strict boreal range in North America. The distributional pattern shows that *A. ciliolatus* occurs only on glaciated terrain. This feature in addition to octaploidy suggests recent origin and, possibly, allopolyploidy. The haploid count of 36 chromosomes was obtained from material collected in northern Minnesota, and meiotic studies indicated regular disjunction of the 36 bivalents.

It seems likely that *A. ciliolatus* arose after hybridization of a heterophyllous aster, such as *A. cordifolius* or *A. sagittifolius,* with a non-heterophyllous type such as *A. simplex* Willd. which contributed the creeping rhizomes and few-headed corymbiform inflorescence that distinguish *A. ciliolatus* from the rest of the HETEROPHYLLI.

Discussion

It can be seen from the foregoing that there is a great deal of complexity in the relationship of the various heterophyllous asters. The group cannot be conveniently studied at one ploidy level since the intricacies of the genome relations interweave through the diploid and tetraploid levels. The diploid species *A. cordifolius, A. sagittifolius,* and *A. texanus* are closely related to one another as evinced by the production of partially fertile F_1 hybrids, but the presence of univalents and subsequent pollen sterility strongly suggest

genetic divergence. The Ozarkian species *A. anomalus* is morphologically, ecologically, and genetically distinct from the other diploids. The status of comparium, or at least cenospecies, is indicated by the failure to achieve successful hybridizations, and by the stimulation to selfing of *A. cordifolius* when crossed with *A. anomalus*. However, there is a great deal of chromosome homology when one considers the regularity of bivalent formation in the diploid F_1 *A. anomalus* ♀ × *sagittifolius* ♂ and quadrivalent associations in the artificial tetraploid F_1 *A. anomalus* ♀ × *shortii* ♂. The distinctness of *A. anomalus* stands in strong contrast to the less well-defined genetic relationship of the other diploid HETERO–PHYLLI.

While *A. texanus, A. sagittifolius,* and *A. cordifolius* might be considered as one species by some, because of their limited ability to exchange genes, the presence of a tetraploid race in the last two species poses an additional complication, especially since it is not known whether they are autotetraploids. The indiscriminate relegation of diploid species to subspecific or varietal rank does not satisfactorily dispense with the problem of the status of the tetraploids. Indeed, it would serve no purpose to alter the nomenclature at the present time.

The tetraploid species furnish the greater stumbling block to taxonomic solution in this group. Fertile species hybrids are produced in several combinations. These F_1 progeny have been carried through the F_2 generation in only two combinations. In both there was reduction in fertility or vigor, or both, which is indicative of some genetic divergence.

Interspecific hybridization among the tetraploid asters yielded less than 25 per cent seed set (Avers, 1953). Germination of hybrid seed was high and the F_1 progeny completely pollen-fertile. Normally outcrossed species are highly heterozygous, and homozygous mostly for dominant genes. Relatively few recessive genes would be retained in the homozygous state unless they were neutral or advantageous to the individual. Recessives may be carried in the genotype despite some disadvantage if they are linked to genes which confer a competitive advantage on the species or individual. When the heterophyllous asters are crossed, many recessive alleles may be present in the homozygous condition in the hybrids, which was not the case in the parental types. With the introduction of increased homozygosity of recessive alleles, hybrid inviability might be manifested in the early death of the F_1 embryo. Thus, reduced seed set in the initial interspecific crosses might be attributed to the elimination of particular genotypes which contain lethal recessive alleles in the homozygous state. The 35 to 45 per cent seed set obtained in intraspecific crosses probably is indicative of lethal and detrimental genes in the species.

Those seeds produced in crosses between tetraploid species germinated to produce vigorous, fertile F_1 hybrids, which suggests that the reproductive barrier obtains prior to embryo maturity. The hybrids which resulted would represent those genotype classes which are highly heterozygous and, homozygous mostly for dominant alleles. The intermediacy of the F_1 generations is probably due to the multiple factor basis of the key taxonomic characters. The production of the F_2 and backcross generations is based on the same premise, i.e., selection of the most heterozygous genotypes and concurrent elimination of those classes which contain many double recessives. Thus, the later hybrid derivatives were also few in number but at the same time, the fertility of many of the segregates was lowered. The decreased fertility might be due to segregation of modifier complexes (Harland, 1936) or to gametes which manifest the effects of cryptic structural hybridity (Stebbins, 1950; Stephens, 1950).

Other factors such as failure of pollen tube growth, genic unbalance, and endosperm disturbances might also be effec-

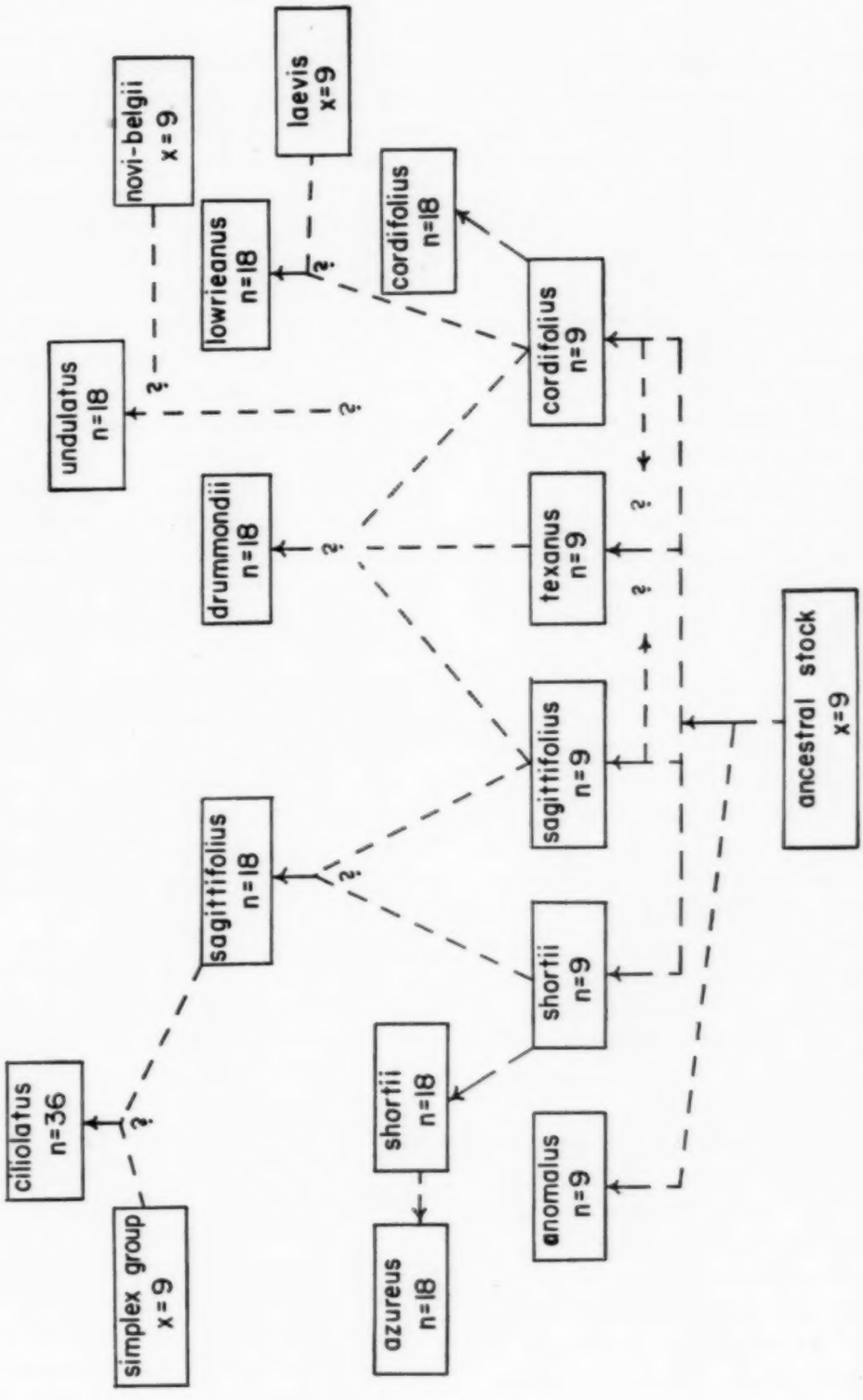

FIG. 2. Phylogenetic pattern of the HETEROPHYLLI.

tive in reducing seed set in interspecific crosses. Although backcrossing is more likely than intercrossing among the few F_1 hybrids in nature, it is possible that the rarity of hybrid swarms in nature is partially due to the elimination of unbalanced genotype classes and selection of those classes which are highly heterozygous, and homozygous for dominant alleles. The close resemblance of the first backcross generation to the recurrent parent (Avers, unpub.) may also be due to such selection of compatible genotypes, as well as to linkage phenomena which increase F_2 variability in the direction of the parental types (Dempster, 1949).

The tentative phylogenetic relationship shown in figure 2 does not do justice to the complexity of the situation. However, it does indicate the difficulties encountered in a series in which auto- and allopolyploidy have played distinctive roles. The group is still actively evolving and the close morphological and genetic relationship indicate that many changes are probably of fairly recent origin. The increased hybridization potential at the tetraploid level has resulted in an array of morphologically intermediate types which tend to blur the species discontinuities at both ploidy levels. *Aster anomalus* is a clear-cut species but *A. texanus, A. cordifolius, A. sagittifolius,* and *A. drummondii* which are very similar morphologically, create many difficulties for the taxonomist.

In spite of the paucity of evidence it is possible to evaluate probable evolutionary trends in this group:

1. Genic differentiation has occurred at both the diploid and tetraploid level as evidenced by the stability of morphological types in the experimental garden. Genic divergence, or perhaps stabilization of different alleles, may be responsible for the elimination of incompatible genotypes in the early embryo stage. Increased incompatibility in interspecific crosses eventually leads to less gametic wastage and, therefore, more successful reproductive isolation (Dobzhansky, 1951).

2. It is evident from the decreased fertility and production of physiologically unbalanced types in later hybrid generations that minute structural changes have occurred among the various genomes. Stephens (1950) assigns a major role in the evolution of cotton species to this phenomenon. The presence of unpaired chromosomes in diploid and tetraploid aster hybrids also is evidence of chrosomal differentiation. As in *Gossypium,* there is less sterility in the tetraploid hybrids than there is in diploids because of the duplication of chromosome material in the former.

3. The extensive distribution of most of the tetraploids indicates that they are indeed established biological entities. Increased stability of these types is accomplished by the absence of multivalent formation. Since many of the tetraploids are probably autopolyploids or segmental allopolyploids, the prevalence of bivalent associations indicates that diploidization has undoubtedly occurred.

4. Ecological isolation enhances reproductive barriers such as hybrid sterility, hybrid inviability, and polyploidy. Although many of these species are sympatric, and may occur in the same population, there are relatively few ecological niches available for hybrids to become established.

Summary

The heterophyllous asters consist of nine taxonomic species, two of which are diploid, three tetraploid, one octaploid, and three which contain both diploid and tetraploid entities. Crosses between the diploid and tetraploid species indicated considerable chromosomal homology, but some structural hybridity was apparent from the presence of univalents and the reduced fertility of hybrid derivatives.

The presence of autotetraploids and segmental allotetraploids, in addition to the diploid species, has resulted in a reticulation of the phylogenetic pattern. It was suggested that interbreeding at the tetraploid level resulted in a chain of intermediates which increased the complexity of

variation in this group. Several barriers to unimpeded gene exchange exist, however, and prevent species amalgamation. Reduced fertility in most diploid F_1 hybrids, degeneration of later hybrid derivatives, reduced viability of F_1 zygotes, zygotic elimination, and ecological differentiation bring about partial reproductive isolation at the homoploid level. As a consequence, widespread intergradation does not occur and the species tend to remain fairly distinct. Localized introgression, however, probably accounts for much of the perplexity of the field botanist.

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LITERATURE CITED

AVERS, CHARLOTTE J. 1953. Biosystematic studies in *Aster*. I. Crossing relationships in the HETEROPHYLLI. Amer. Jour. Bot. In press.

CLAUSEN, J. 1951. Stages in the evolution of plant species. Cornell Univ. Press. Ithaca.

CLAUSEN, J., D. D. KECK, AND W. M. HIESEY. 1940. Experimental studies on the nature of species. I. Effect of varied environments on western North American plants. Carnegie Inst. Wash. Pub. 520.

CLAUSEN, R. E., AND D. R. CAMERON. 1944. Inheritance in *Nicotiana tabacum*. XVIII. Monosomic analysis. Genetics, **29**: 447–477.

COLLINS, J. L., L. HOLLINGSHEAD, AND P. AVERY. 1929. Interspecific hybrids in *Crepis*. III. Constant fertile forms containing chromosomes derived from two species. Genetics, **14**: 305–320.

DARLINGTON, C. D. 1930. Studies in *Prunus*. III. Jour. Genetics, **22**: 65–93.

DARLINGTON, C. D., AND E. K. J. AMMAL. 1945. Chromosome atlas of cultivated plants. George Allen and Unwin. London.

DEMPSTER, E. R. 1949. Effects of linkage on parental-combination and recombination frequencies in F_2. Genetics, **34**: 272–284.

DOBZHANSKY, TH. 1951. Genetics and the origin of species. 3rd ed. Columbia Univ. Press. New York.

FERNALD, M. L. 1902. *Aster undulatus* × *novi-belgii*. Rhodora, **4**: 186–187.

HARLAND, S. C. 1936. The genetical conception of the species. Biol. Rev., **11**: 83–112.

HOWARD, W. H., AND I. MANTON. 1946. Autopolyploid and allopolyploid watercress with the description of a new species. Ann. Bot., **10**: 1–13.

LJUNGDAHL, H. 1924. Über die herkunft der in der meiosis konjugierenden chromosomen bei *Papaver*-hybrids. Svensk. Bot. Tidskr., **18**: 279–291.

MANTON, I. 1950. Problems of cytology and evolution in the Pteridophyta. University Press, Cambridge.

MENZEL, MARGARET Y. 1950. Cytotaxonomic observations on some genera of the Solanae: *Margaranthus*, *Saracha*, and *Quincula*. Amer. Jour. Bot., **37**: 25–30.

——. 1952. Polygenomic hybrids in *Gossypium*. III. Somatic reduction in a phenotypically-altered branch of a three-species hexaploid. Amer. Jour. Bot., **39**: 625–633.

MENZEL, MARGARET Y., AND META S. BROWN. 1952. Polygenomic hybrids in *Gossypium*. II. Mosaic formation and somatic reduction. Amer. Jour. Bot., **39**: 59–69.

MÜNTZING, A. 1941. New material and cross combinations in *Galeopsis* after colchicine-induced chromosome doubling. Hereditas, **27**: 193–201.

SHINNERS, L. H. 1941. The genus *Aster* in Wisconsin. Amer. Midl. Nat., **26**: 398–420.

——. 1950. Notes on Texas Compositae. V. Field and Laboratory, **18**: 32–42.

SMALL, J. K. 1913. Flora of the southeastern United States. Rev. ed. *pp.* 1206–1228.

STEBBINS, G. L., JR. 1950. Variation and evolution in plants. Columbia Univ. Press. New York.

STEPHENS, S. G. 1950. The internal mechanism of speciation in *Gossypium*. Bot. Rev., **16**: 115–149.

EGG SIZE IN ARCTIC, ANTARCTIC AND DEEP-SEA FISHES

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General Considerations and Data

In a recent review of the reproductive and larval ecology of marine, bottom-living invertebrates, Thorson (1950) has shown that there are striking parallels between those found in the arctic, antarctic and deep-sea regions. Certainly in the arctic and most probably in the other two areas, a high proportion of the invertebrates produce large, yolky eggs by comparison with related species from other environments. Equally, Thorson has shown that in each of these three faunal areas there has been a marked suppression of the pelagic phase in development.

Arctic, antarctic and deep-sea fishes also produce relatively few, large, yolky eggs, while there is evidence from the circumpolar seas that the larvae are hatched at comparatively advanced stages. It is the present intention to bring together data on egg sizes and the lengths and forms of newly hatched larvae found in these major areas, to compare these with the sizes of eggs and larvae of related species from neighboring regions and then to discuss how far these differences may be correlated with environmental conditions. For the invertebrates Thorson (1950) has given a stimulating analysis along such lines.

Arctic fishes: Complete data on the arctic fish fauna will not be given here. Rass (1941) gives tables of all the available measurements of eggs and early larvae in arctic, subarctic, arctic-boreal and boreal species of the Gadidae, Cottidae, Zoarcidae, Liparidae, Agonidae, Cyclopteridae and Anarrhichadidae. On the basis of this work he has proposed certain ecological rules which will be discussed later, together with the data for the antarctic and deep-sea species. His summarized data for the first four families are listed in table 1.

Antarctic fishes from the coastal zones: In only one species (or two, if Bouvet Island is included in the Antarctic region) are the sizes of the free eggs known. The measurements of all others have been made from an examination of the ovaries. These are indicated (table 2) by the plus sign following the dimensions, indicating that the eggs are at least of this size, since there is likely to be an increase in the diameter of the eggs after fertilization and laying.

Measurements (or data) on ripe or ripening ovarian eggs only have been taken, this being particularly indicated by the orange-yellow or brownish-yellow color of such eggs. Immature eggs always appear to be whitish, which can be clearly seen in ovaries containing more than one generation of eggs. The measurements are all in millimeters.

Finally it may be conveniently noted here that judging from the distribution of the early larval stages, which have been taken almost entirely in coastal waters, and the finding of the eggs of *Harpagifer bispinis* in shallow water at Port Lockroy, Palmer Archipelago and those of a notothenid near Bouvet Island (in 119 to 124 meters), the coastal fishes as far as known lay their eggs in relatively shallow waters.

Since the tendency of arctic fishes to produce relatively large, yolky eggs has been considered by Rass (1941), it will only be necessary to discuss the data for antarctic species.

Comparing representatives of the genus *Notothenia* from the Falkland Islands with species of other notothenioform genera liv-

TABLE 1. *Arctic and northern fishes (data from Rass (1941))*

Group of species	Egg diam. mm.	Size of newly hatched larvae mm.	No. of eggs produced	Maximum body size mm.
		Liparidae		
Arctic and chiefly arctic	2.13–4.59	7.2–16.0	300–434	172–270
Arctic boreal	1.35–1.67	ca 5.4	—	275
Boreal	1.03–1.19	3.3–3.8	793	125
		Cottidae		
Arctic	1.8 –4.0	9.0–11.5	57–680	115–325
Arctic boreal	1.95–2.51	7.4– 8.6	2,742	600–1,000
Boreal and north boreal	1.51–2.04	4.4– 5.5	1,586–28,735	54–165
		Zoarcidae		
Arctic (inc. high arctic and panarctic)	3.5–8.7	25–27	17–500	125–530
Sub-arctic	6.0	—	1,200	745
Boreal	ca 3.0	ca 3.7	86–400 (1800)	610
		Gadidae (Gadini)		
Arctic	(1.4)1.5–1.9(2.08)	4.3–5.3	20,000–67,000	320–560
North boreal	1.13–1.67	(3)3.7–4.0	170,000–9,344,000	970–1,800
South boreal and endemic boreal	0.97–1.32	3.0–3.8	61,686–8,260,000	250–1,200

ing in the antarctic area, it will be seen that the latter in nearly all instances (and probably in all, when it is remembered that most measurements are of ovarian eggs) produce relatively larger eggs. It is unfortunate that within the genus *Notothenia,* where closer comparisons could be made, the data are rather inadequate. However, the antarctic species, *N. nudifrons,* which reaches a maximum recorded length of 220 mm. produces eggs at least 2 mm. in diameter, whereas *N. squamiceps* and *N. sima* from the Falkland Islands lay eggs which attain diameters of 1.25–1.4 and 1.2–1.4 mm. respectively. As these figures stand, the diameters of the eggs of all three species bear much the same relationship to the maximum known sizes, but it is likely that the free eggs of *nudifrons* will be more than 2 mm. in diameter. (It should also be added that there is no constant ratio, even comparing related species, between the size attained and the size of the eggs, which Rass (1941) has emphasized in his analysis of arctic species.)

It may thus be concluded that, just as in the arctic fish families, there is a general tendency within the *Nototheniiformes* for antarctic species to produce larger, more yolky eggs than their nearest relatives from warmer seas.

Deep-sea fishes: Here the data have been mainly taken from Sanzo (1931 and 1931a). Data for the first three species (table 3) are from Alcock (1899); for *Nessorhamphus* from Schmidt (1930); and for a deep-sea eel, from Beebe (1936). The measurements of the ovarian eggs of *Opisthoproctus grimaldii* and *Scopelarchus elongatus* were made by the writer. Figures for the diameter of the vitellus or yolk sphere have been included wherever the egg has a large perivitelline space.

In addition to the above statistics Tåning (1918), Beebe and Vander Pyl (1944) and Johnsen (1944) have measured the diameters of ovarian eggs in a number of species of myctophid fishes, while Hjort (1912) and Jespersen and Tåning (1926) have data for *Cyclothone* spp. In the myctophids the diameters of ovarian eggs, with differing degrees of ripeness, ranged from 0.25 to 0.6 mm. for specimens measuring from 20 to 150 mm.

TABLE 2. *Antarctic fishes*

(Except for *Austrolycichthys* and *Paraliparis*, which belong to the Zoarcidae and Liparidae, respectively, the fishes in this table are classified in the Nototheniiformes.)

Species	Egg diam. mm.	Length of newly hatched larvae mm.	Month recorded	Max. size of adults mm.	Authority
Notothenia nudifrons Lönnberg	1.5– 2.0+ Egg No. 2–3,000	—	—	220	Marshall
Notothenia coriiceps Richardson	2.5 –3.0+	—	Aug.	540	Marshall
Notothenia rossii Richardson	2.25–3.0+	—	Mar.	850	Lönnberg (1906)
Nototheniid sp.* prob. *Notothenia*	1.9 –2.2	5.6–5.8	Oct.	—	Marshall
Trematomus bernacchii Boulenger	3.8+	—	Aug.	322	Waite (1911)
Pleuragramma antarcticum Boulenger	—	6.0	Dec.	200	Regan (1916)
Artedidraco mirus Lönnberg	3.0+	—	May	115	Lönnberg (1905)
Artedidraco skottsbergi Lönnberg	2.5 –3.0+	ca 13.0	Jan.	120	Regan (1916)
Artedidraco loennbergi Roule	2.5 –3.0+	—	Jan.	110	Regan (1916)
Harpagifer bispinis (Schneider)	2.4 –2.6	8.6–8.8	Nov.	104	Marshall
Bathydraco nudiceps Waite	2.6+	—	Jan.	144	Waite (1916)
Gerlachea australis Dollo	2.5 –3.0+	—	Jan.	208	Roule, Angel & Despax (1913)
Champsocephalus gunnari Lönnberg	3.0 –4.0+	—	May	436	Lönnberg (1905)
Pagetopsis macropterus Boulenger	4.0+	ca 14.0	May	288	Pappenheim (1912) Regan (1916)
**Chaenichthys rugosus* Regan	3.0 –4.0+	—	Oct.–Feb.	400	Regan (1916)
Chionodraco kathleenae Regan	4.0 –4.5+	—	Oct.–Feb.	500	Marshall
Austrolycichthys brachycephalus (Pappenheim)	5.0+	—	—	280	Waite
Paraliparis gracilis Norman	2.6 –2.9	9.4–9.5	Apr. (S. Georgia)	115	Marshall

* Distribution: Kerguelen Island.

For comparison with the above data (which except for the last two species concern notothenioform fishes), measurements are given below of *Notothenia* species from the Falkland Islands.

Notothenia squamiceps Peters	1.25–1.4	—	May	120	Marshall
Notothenia sima Richardson	1.2 –1.4	—	Nov.	140	Marshall
Notothenia sp.	1.5	6.0	Sept.	—	Regan (1916)
Notothenia sp.	1.7	—	May	—	Regan (1916)

* Taken by "Discovery" Investigations just off Bouvet Island.

TABLE 3

Species	Egg diameter mm.	Length of newly hatched larvae mm.	Maximum size of adults mm.
Alepocephalus bicolor Alcock	4.0+	—	298
Rouleina güntheri (Alcock)	2.0–3.0+	—	152
Lepogenys squamosus (Alcock)	3.0+	—	260
Argentina sphyraena L.	1.60–1.68	ca. 7.0	357
Argentina silus (Ascanius)	3.0 –3.5	7.7	460
Argentina leioglossa (Valenciennes)	1.44–1.52	ca. 5.0	200
Microstoma microstoma (Risso)	1.6 –1.72	ca. 6.0	91.0
Nansenia oblita J. Schmidt	1.48	4.0	180.0
Opisthoproctus grimaldii Zugmayer	1.0 –1.5+	—	64.0
Argyropelecus hemigymnus (Cocco)	0.92–1.04	2.5	45.0
Ichthyococcus ovatus Cocco	0.80	2.2	45.0
Maurolicus muelleri (Gmelin)	1.32–1.58 Vitellus ca. 1.0	3.0	65.0
Gonostoma denudatum Rafinesque	0.8 –0.81+	ca. 6.0	172.0
Vinciguerria attenuata (Cocco)	0.84–0.92+	—	43.0
Diaphus gemellari (Cocco)	1.64–1.72	4.0	113.0
Chauliodus sloanei Bloch-Schneider	2.24–2.52 Vitellus 1.4	7.2	270.0
Scopelarchus elongatus Norman	0.6 –1.0+	—	200.0
Nessorhamphus ingolfianus J. Schmidt	2.40–2.70 Vitellus 1.20–1.65	7.0	248.5
A deep-sea eel	3.50 Vitellus ca. 1.75	11.0	—
Coelorhynchus coelorynchus (Risso)	1.15–1.21	4.2	380
Macrurus sclerorhynchus Valenciennes	1.6	4.28	325

in length. Of particular interest is the estimated number of eggs in the two ovaries which was from about 100 to 3300. For *Cyclothone signata* of length 30 to 35 mm. (which Jespersen and Tåning (1926) think was probably *C. braueri*) and *C. microdon* of about 60 mm. Hjort gives diameters of 0.46 and 0.56 respectively, while in the former species he found about 1000 eggs in the ovaries and in the latter 10,000. In *Cyclothone braueri* of 25 mm. Jespersen and Tåning found 260 eggs whereas in a *C. microdon pygmaea* of 24 mm. there were about 200 eggs. In both species the eggs measured about 0.3 mm. in diameter.

For the deep-sea fishes it is not possible to compare the data for the species in table 3 with those for closely related species from shallower waters. In this instance some measure may be obtained of the marked trend towards large eggs by comparing the first eight with a family of inshore pelagic fishes from the same order. Here the measurements given for clupeid species of Ehrenbaum (1936) and Sanzo (1931) for the North Atlantic and Mediterranean, and Delsman (1923–34) for species from the East Indies shows that in nearly all instances the eggs of the deep-sea fishes are relatively larger and, wherever the comparison can be made, much fewer in number.

An apparent objection to the latter statement could be that since many deep-sea fishes are small they must needs produce few, seemingly large eggs, in order that a larva of adequate size should result. However, many species of fishes from coastal waters maintain their populations by means of eggs which give rise to larvae of no more than 1.5 mm. or so in length (for example, see Hildebrand and Cable (1930 and 1938)).

The Ecological Significance of Large Eggs and Larvae in Circumpolar and Deep Oceanic Waters

Before considering the above data, the conclusions reached by Thorson (1950) and Rass (1941) will be briefly reviewed. Thorson, when writing of arctic and antarctic invertebrates, has particularly stressed the sparse phytoplankton populations of the inshore waters of these regions, which are moreover limited in time. Evidence to support this comes from his (1936) work in East Greenland waters and that of Hardy (1938) and Hart (1934) in sub-antarctic and antarctic waters. Thorson's (1936) arctic investigations showed that in the fjords and coastal waters of East Greenland a limited phytoplankton production was restricted to 1–1½ months in the summer. Charts of the phytoplankton around South Georgia and the South Shetlands given by Hardy (1928) show that production must be poor in the coastal waters while Hart (1934) who carried out a detailed survey of Cumberland Bay, South Georgia found a relatively poor phytoplankton, limited to the period from November to January when the main production consists of two flowerings lasting 3 and 2 weeks respectively. Furthermore, later work by Hart (1942) has clearly shown that the period of phytoplankton production is even more restricted in high antarctic waters, the graph on page 315 indicating a sudden outburst during January, rising to a maximum in February.

This shortening of the phytoplankton season is attributed to the more severe ice conditions, for ". . . large scale production can only begin when the first large areas of open water are formed in January, and as new ice begins to form in March it follows that the annual production must be crowded into three summer months with no possibility of a secondary autumnal increase." Regarding the quantities of phytoplankton in immediate coastal waters little can be said since the survey was not concerned with these areas. Thorson (1950) contends, however, that the conditions suggested by Hart (1934) limiting phytoplankton production in Cumberland Bay, South Georgia (photosynthesis restricted by turbulence due to high winds and waters clouded particularly by morainic mud), should also prevail in the inshore waters of other antarctic coasts. A sparse phytoplankton would be expected.

Finally, the bold generalized picture of the variation of the biomass of plankton with latitude given by Zenkevitch (1949) may be included. The graph on page 524 shows very low values in high arctic and antarctic waters. Zenkevitch concludes that: "Les regions polaires, en raison des glaces qui les recouvrant durant la majeure partie de l'annee, de leur regime lumineaux defavorable, du manque de matieres nutritives et de la faible circulation verticale (dans l'Arctique) ne sont pas propices a un riche developpment de la vie."

From such considerations Thorson (1950) reasonably argues that the larval invertebrates, which are dependent on the phytoplankton for food, must complete their development from hatching to metamorphosis during the brief, scanty flowering of the phytoplankton and at temperatures between 1°–4° C. He concludes that the majority of invertebrates have been unable to produce pelagic larvae capable of developing under such exacting conditions; hence the high percentage of species with non-pelagic development. One significance of large yolky eggs in the polar environments is ". . . that the greater the size of the individual when born, the smaller will be its relative food requirements and the better its chance of competing under poor food conditions." Concerning the bottom-living, deep-sea invertebrates, Thorson again emphasizes poor quantities of food and low temperatures coupled with the fact that the larvae would have to migrate vertically through a great height of water to reach the surface living phytoplankton; thus again the

need for non-pelagic development (and presumably for relatively large eggs).

In working on much the same problems Rass (1941) has considered temperature to be the dominating factor. He has summed up his findings in the following rule: "The size of eggs of aquatic animals is in accordance with their breeding temperatures. In series of related species the size of the eggs is inversely proportional to the temperature of the medium at the moment of egg laying, i.e., the eggs being greater, the nearer to the pole lies the habitat of the species." The increased egg size and longer periods of development are thought to be adaptive allowing the embryo ". . . to develop at rates corresponding to the peculiar conditions of arctic seas." The greater sizes of the eggs are considered to arise by dissimilar rates of growth and differentiation at low temperatures, the latter rate being retarded relative to the former. Rass concludes that species that were unable to adapt the rates of development to these environmental conditions were weeded out in the struggle for existence.

It will be seen that these conclusions are rather different from those of Thorson. While both workers consider that selective processes have been responsible for these parallelisms, Thorson has taken into consideration biological as well as physical factors, while Rass has been more impressed with temperature conditions. Here the view will also be taken that larger eggs and larvae have come into being as the result of selection, and on such a basis the likely advantages of larger eggs and larvae will be considered in relation to what is known of the environmental conditions.

Arctic and antarctic fishes: Concerning arctic and antarctic fishes living in the coastal zones it will now be necessary to consider the type of egg produced. Of the arctic gadoids dealt with by Rass (1941) one, *Gadus* (*Boreogadus*) *saida* produces pelagic eggs but *G.* (*Eleginus*) *navaga* lays demersal eggs,[1] while in the Cottidae, the Zoarcidae, the Anarrhichadidae, the Liparidae, the Cyclopteridae and the Agonidae, the eggs of all species are almost certainly demersal. In the Antarctic the eggs of *Harpagifer bispinis* and *Paraliparis gracilis* are definitely demersal and so are those of *Notothenia sima* and *N. squamiceps* from the Patagonian-Falkland region. The writer has also found the stomachs of Weddell seals packed with the eggs of antarctic nototheniids, no doubt obtained by the seal scooping up the egg masses attached to the sea floor (it is difficult to imagine how such large quantities of eggs could be swallowed if they were floating). It may thus be concluded that the nototheniiform fishes lay demersal eggs (with the possible exception of the deep-water species, *Pleuragramma antarcticum*).

It is well known that demersal eggs are usually larger than pelagic eggs (which does not, of course, invalidate the conclusions reached earlier concerning the trend towards larger eggs in the arctic and antarctic, since the species compared all produce demersal eggs). What is of some interest is whether the fishes with demersal eggs invariably guard them during the incubation period. It would be reasonable to expect such fishes to produce fewer and larger eggs than those shedding pelagic eggs, where wastage of eggs and larvae is enormous. In the nototheniiform fishes parental care may well occur in one species, *Notothenia sima*. A specimen of this fish collected by Vallentin in the Falkland Islands was found coiled around a mass of eggs. In arctic fishes parental care is known in the Cottidae, Agonidae and Cyclopteridae, while it is also interesting that in the list given by Breder (1943) of fishes producing demersal, non-spherical eggs, all (except the Ammodytidae) are known to guard them.

Whether demersal eggs are guarded or not, it appears that in these and pelagic

[1] Pelagic eggs float freely in the sea; demersal eggs rest on or are attached to the sea floor.

eggs evolution has occurred along opposite lines. In species shedding pelagic eggs some compromise would appear to be struck between ensuring sufficient food reserves for the developing embryo (and newly hatched larva) and a large production of eggs to ensure brood survival. In species laying demersal eggs the balance is between the production of a sufficient number of eggs to ensure survival and the advantages of laying large eggs giving rise to large larvae with a good start in life. However this may be, it is of particular interest that practically every species of bottom-living fish from the coastal zones of the arctic and antarctic produces demersal eggs.

The advantages of larger eggs (and hence of larger larvae on hatching) in arctic and antarctic seas would seem to be as follows:

1. As already mentioned, Thorson (1950) referring to work by Zeuthen (1947) makes the point that the greater the individual when born, the smaller its relative food requirements.

2. The larger the larva the faster it will be able to swim, and the greater will be its chances of finding the right kind of food over a given period of time.

3. The larger the eggs, the fewer the female can produce in a limited coelomic space, and hence the less should be intraspecific competition for food among the larvae.

4. The larger the larva of one species relative to that of another, the greater will be its advantages in securing food.

5. Associated with the evolution towards larger egg size, there has been a tendency for the larva to be hatched at a comparatively advanced stage. This would give it a "better start in life."

Taking these statements in order, Thorson (1950) has pointed out the advantages of (1) in polar coastal areas where production of phytoplankton (and hence of zooplankton) is limited in quantity. The second assertion of extra swimming speed and the third of reduced intra-specific competition would also confer advantages where food is limited. As Harvey (1950) has recently emphasized, a critical period in the life of a larval fish is when the yolk reserves have been exhausted and it begins to feed on small zooplankton organisms. Mortality will be heavy unless sufficient food of suitable size can be obtained. Here extra size may mean that the larva is less restricted, for example, as to the size range of copepods it can manage to eat.

Concerning the advantage of size in interspecific larval competition, there is little quantitative evidence on which to base any judgment. Interspecific competition could, however, be particularly intense. All reliable evidence such as the finding of the egg masses of *Harpagifer bispinis* or the time of capture of newly hatched larvae points to a main summer spawning period for Antarctic coastal fishes. (The eggs in the stomachs of Weddell seals were found in November and early January, while none were seen in seals killed during the spring, autumn and winter.) If the larvae of all species appear together in the sparse plankton during its short productive period, there might be severe competition for food. For example,[2] in Cumberland Bay, South Georgia, which has been well explored by the scientific staff of Discovery Investigations, seventeen species have been recorded. If the larvae of all species fed on the same food then extra size would be of importance in the struggle for survival. Perhaps this is so if the competing larvae are of closely allied species. Comparison, however, of two dissimilar larvae such as *Pleuragramma antarctica* and *Pagetopsis macropterus,* which occurred together in a haul taken by Discovery Investigations in the Ross Sea, shows how dissimilar larval feeding habits can be. In this haul are hundreds of the former species together with a few of the latter. The *Pleuragramma* were mainly feeding on small

[2] Conditions in the Antarctic may be contrasted with those in the North Sea, where there is a succession of spawning.

copepods and were in turn being preyed on by the larval *Pagetopsis*. For example, in the stomach of a larval *Pagetopsis* of about 14 mm. were found two larval *Pleuragramma* of about 10 mm. in length.

Regarding the hatching of arctic fishes, Rass (1941) has shown that the early larval stages with yolk vesicle and embryonic fin-fold tend to be dropped out of the pelagic phase. The data on antarctic larval fishes from Regan (1916) and the observations of the writer show that the newly hatched larvae have a well-formed mouth and that the larvae of the Chaenichthyidae resemble certain boreal gadoids in the early development of the pelvic fins. As in the arctic, the larvae are hatched with a relatively small yolk sac, this being the largest in *Pagetopsis macropterus* and *Artedidraco skottsbergi,* but even in these species the jaws are formidable and the mouth capacious. Of particular interest are the newly hatched young of *Paraliparis gracilis,* which have the fully-developed complement of dorsal, anal, upper pectoral and caudal rays, the tip of the notochord being upturned with two small hypural bones plainly differentiated.[3] This presents the possibility that certain species may have no pelagic stages (it requires little change to turn this "larva" into an adolescent). In the arctic no pelagic larvae of the deep-living genus *Lycodes* have been found, while Burke (1930) observes that the same is true of the deep-sea liparids. However, the eggs of *Paraliparis gracilis* were found between the relatively shallow depths of 120 and 204 meters off Cumberland Bay, South Georgia. Of other nototheniids with larval stages found in inshore waters, *Pagetopsis macropterus,* when adult, has been taken between 200 and 655 meters, *Pleuragramma antarcticum* between 0 to 500 meters, *Artedidraco skottsbergi* between 40 and 380 meters, and *Chionodraco kathleenae* between 90 and 600 meters. The remainder when adult live in shallower waters. Perhaps the fishes from intermediate depths make an inshore migration to spawn, but it is quite possible that the deeper living forms such as the species of *Bathydraco,* the zoarcids and liparids have no pelagic larvae. If this is so it is an interesting parallel with a great many species of invertebrates.

Apart from the possible dispensing with a pelagic phase, the hatching at comparatively advanced stages would seem to have a two-fold importance to pelagic larvae in polar waters. Firstly, the larvae are able to feed at once on zooplankton organisms, a quicker means of getting nourishment than feeding on thinly concentrated phytoplankton (and, as already mentioned, a large and well advanced larva is less restricted as to the sizes of zooplankton organisms it can seize). Secondly, it is better able to complete the critical stages of metamorphosis during the short period of plankton production. It would seem essential that the young fish should be well advanced by the time the formation of sea ice and the decreasing daylight begin seriously to limit photosynthesis. What happens to the fry during the autumn and winter is not clear. In the Antarctic they do not appear to be present in inshore waters, since I found none in the stomachs of adult nototheniids (about 1000 were examined), caught by hand-line through ice-holes at Hope Bay, Grahamland. It may be that they spend these seasons in offshore waters still living pelagically and feeding on planktonic organisms. There is little evidence to support this suggestion, but Pappenheim (1912) does record the young of *Pagetopsis macropterus* (length 32 mm.) in April at the "Gauss" Winter Station, which were taken in a young fish net worked down to 350 meters. It is also of interest that both *Notothenia coriiceps* and *N. rossii* have sleek, silvery pelagic fry quite unlike the large-headed adults.

Turning now to the advantages of large eggs and larvae in relation to the physical

[3] For arctic fish Rass (1941) notes that the rays of the unpaired fins are formed during the egg stage in *Cyclopteropsis macalpini, Eumicrotremus spinosus,* species of *Lycodes* and *Lycenchelys* and in *Gymnacanthus.*

conditions of polar seas, the following suggestions are made.

1. Carter (1940) has pointed out that the larger the organism the greater the degree of thermal insulation from the environment. In high polar waters where the temperature never rises much above 0° C. this may well be of importance. It is known (e.g., see Zeuthen, 1947) that larvae have a much higher metabolic rate than adults; consequently it is likely to be an advantage for them to be hatched with as low a surface : volume ratio as possible to counteract too rapid a loss of metabolic heat into the surrounding waters.

2. It has often been shown that within a species and within certain limits the rate of development is directly correlated with temperature. Gray (1928) showed this very clearly for the trout; and that larvae hatching from eggs incubated at lower temperatures were significantly larger than those hatching at higher temperatures. Moreover, he found direct correlations between the amount of yolk, the specific growth rate of the embryo and the final size of the embryo. In poikilothermous animals the effect of polar temperatures could be to slow up the rate of development to such a degree that the larvae would be unable to take full advantage of the short seasonal outburst of the coastal plankton. Gray's work could suggest that an increased supply of yolk would have the effect of counteracting the retarding effect of low temperatures on development. Moore (1949) working on the genus *Rana* found that egg size in northern species tended to be greater than in southern species. Particularly interesting is his finding that, except at high temperatures, the embryos of the northern species have a more rapid rate of development than the southern species. This compensates for the retarding influence of the lower northern temperatures. There is, however, little comparable evidence from related species of fish and the effect of extra yolk on embryonic growth rates will not be considered further.

3. The size of the larva on hatching is directly correlated with the size of the egg. There does appear to be evidence in support of the suggestion of Dahl (1912) and others that the larger the larva on hatching the quicker the growth rate (at least within a particular species). Brown (1946) working on trout found that the predominant factor influencing growth of the fry was the size of an individual relative to its neighbors in the tank, the larger fry growing faster than the smaller. Whether this factor would be of importance in the sea is not known. However, if it is concluded that greater size leads to more rapid growth this would again be of significance in counterbalancing the retarding effect of low temperatures.[4] There can be no doubt that compensating factors are involved. Thorson (1950) found that the time for pelagic development is much the same for tropical and northern representatives of a given invertebrate genus (although within a particular area differing temperature conditions have a marked effect on the duration of pelagic phases of a given species. It is evidently not safe to take the results of experiment on one species, and on this basis generalize about other species (even if closely related) in other environments.

Viscosity as a physical factor is difficult to assess. Firstly, it has an impeding effect on swimming movements, and secondly, it retards the rate of sinking, which is also governed by the size and form of the organism. Average sea water (of salinity 35‰) at a temperature of 0° C. has roughly double the viscosity of water at 25° C. Comparison of the larva of an arctic or antarctic fish with that of a bathypelagic species living in tropical or subtropical waters shows clearly the twofold effect of viscosity. While the two former must expend considerable energy in overcoming the impeding effect of the medium (this counteracting to some degree the

[4] There is another factor which should be mentioned here. Castle (1932) has argued that there is likely to be selection for rapid larval growth and robustness where food and space are limited (the latter not, of course, applying in the sea).

advantage of increased swimming speed associated with extra size), it requires relatively little expenditure to maintain its position in the water column: for the bathypelagic fish larva the situation is reversed. Concerning the decreased surface: volume ratio in larger larvae, this would lead to an increase in the rate of sinking but the larva in colder waters would be at an advantage. Clearly experiments would be necessary to elucidate these relationships. In sea water of salinity 35.01‰ at a temperature of 18.5° C. Gardiner (1933) found that an anesthetized *Calanus finmarchicus* of length 4.0 mm. sank at nearly six times the rate compared to an individual of length 2.1 mm.

Deep-sea fishes: Finally environmental conditions in oceanic regions will be considered in relation to egg size in deep-sea fishes. Here the recent work of Riley, Stommel, and Bumpus (1949) is of interest. They have analyzed plankton interrelationships in different regions of the western North Atlantic; in coastal waters, Georges Bank, Slope water, Gulf Stream water and in the Sargasso Sea. The latter only need be considered. Briefly their conclusions are as follows: (1) Production of phytoplankton in the Sargasso Sea may not be much less than that of coastal and bank waters throughout a year. There appears to be no pronounced maxima and minima in the populations but these are reduced during the summer period of maximum stability, being followed by an increase in autumn or early winter. Lastly the phytoplankton has a greater vertical spread in the clear waters of the Sargasso Sea. (2) The estimated mean annual crop of zooplankton in the Sargasso Sea (measured from 0–900 meters) is about the same as that in coastal areas (with a water column of from 40–200 meters).

Conditions in the ocean basins have been well characterized by Riley, Stommel, and Bumpus (1949) who conclude that in comparison with coastal or bank areas ". . . deep oceanic waters, such as the Sargasso Sea, may be only slightly less productive of plant material, but because of the extended disposition of phytoplankton in the vertical column, more effort must be expended to eat it, leading to a larger fraction of loss by respiration at each trophic level and a progressive attenuation of higher members of the food chain."

It is sometimes assumed that deep-sea fishes spawn in deep water although Heyerdahl and others (1950) appear to have taken deep-sea fish eggs in the surface layers.[5] However this may be, the young larvae are found in the upper layers of the sea, where the greater concentrations of plankton are nearly always found. (In the eastern tropical Pacific Jespersen (1935) found greater concentrations of macroplankton at the deeper levels.) Spawning in certain species may be more intense during certain times of the year, while in others there appear to be no seasonal rhythms. In the Mediterranean Sanzo (1931) found most eggs during the winter months, while Tåning (1918) and Jespersen and Tåning (1926) found evidence of a seasonal occurrence of young larvae in certain gonostomatid and myctophid fishes, although in some it looked as though spawning went on throughout the year.

Compared to arctic and antarctic coastal fishes, the figures of deep-sea species given by Sanzo (1931) show that the newly hatched larvae have a relatively larger yolk sac and that in general they are less well advanced in development. They are none the less large relative to the adult size. However, as already stated, there appear to be no pronounced maxima and minima in oceanic plankton, so that at any time (except perhaps during the height of summer) planktonic food is present in much the same concentrations. There would appear to be no need to take rapid advantage of a short flowering of the phytoplankton as in polar areas.

When the yolk reserves have been used up the larvae are well advanced with well

[5] The eggs may, of course, rise to the surface after being shed at deeper levels.

developed jaws. Examples of the lengths they have reached at this stage are: *Argentina leioglossa* 6–7 mm., *Microstoma microstoma* 6–7 mm., *Maurolicus muelleri* 4.0 mm., *Chauliodus* 8–9 mm., and *Argyropelecus* 4–5 mm. At these sizes they probably begin to feed on small copepods.

The number of the latter organisms per unit volume will be small, not necessarily because of limited productivity but more perhaps on account of the great vertical spread of the plankton. Bearing in mind the other conclusions of Riley and his colleagues quoted earlier, certain advantages of large eggs giving rise to large larvae can be appreciated. In oceanic waters these would seem to be: (1) The larger the larvae the less the relative food requirements. (2) The greater the size the faster the speed and hence the better the chances of finding swimming food of a suitable size.

Intra-specific and inter-specific larval competition for food cannot be considered here. Perhaps if the larvae occur in schools, the fact of there being relatively few eggs laid per female may be significant. Similarly, the possible correlation between the size on hatching and subsequent growth rate will not be discussed beyond again referring to the conclusions of Castle (1932) concerning selection for rapid larval growth where food is limited. existence of adaptation.

Lastly, since by far the greater number of deep-sea fish species are found between latitudes 50° N. and 50° S. and since the majority spend their early larval life in waters which are warm or very warm compared to the Arctic or Antarctic, the earlier arguments concerning extra size in low temperatures have no place here.

Discussion

It will be evident that in trying to appreciate the factors which have conditioned the increase in egg size of polar and deep-sea fishes, Thorson's (1950) approach has been followed rather than that of Rass (1941). As the former has emphasized, knowledge of the physical environment provides but part of the picture; what is required is a knowledge of the means whereby a marine species manages to maintain its populations over a range of environmental conditions, both physical and biological.

The ecological rule proposed by Rass that the size of eggs of aquatic animals is inversely related to the breeding temperatures is an oversimplification. It would not be difficult to find exceptions to this sweeping generalization. While it is not proposed to consider instances one may be taken which seems to be more intelligible on an ecological basis than on a purely physical approach.

Data from Bruun (1935), Breder (1932 and 1938) and Hubbs and Kampa (1946) show that the oceanic species of flying-fishes produce significantly larger eggs than the neritic species,[6] these differences not being correlated with adult sizes. As far as can be judged these data also show that the oceanic species have a very extended breeding season, whereas in neritic species this is limited to definite periods in the year. Since it may reasonably be assumed that the larger oceanic eggs will produce larger larvae, this may well be linked with feeding conditions. As already indicated the concentration of plankton per unit volume in oceanic waters is for the most part a good deal less than that of neritic waters. Thus in parallel with deep-sea fishes the extra size of oceanic flying-fish larva on hatching enables it to secure more effectively the necessary food for maintenance and growth.

If the foregoing has indicated the pitfalls of formulating ecological rules without comprehensive data on ecology and physiology, no better example of this could be quoted than the variation of plate number in the stickleback, *Gasterosteus aculeatus*. Previous to the work of Heuts (1947) it was thought that plate number decreased regularly and continuously from

[6] Neritic species are those that live in the relatively shallow, coastal waters which overlie the continental shelf.

north to south and from waters of high salinities to those with low ones. Heuts showed that this was only partly true and in particular that there were two types of populations in certain areas, one with a high number of plates occurring in the vicinity of the sea and the other with lower numbers living in fresh waters. Correlating with these differing plate numbers were differences in the physiology of the adults and the eggs. As might be expected (but which required proof), eggs from individuals with high plate numbers needed different conditions of temperature and salinity for successful hatching than those from individuals having fewer plates. Of particular interest for the present paper are these concluding remarks: "The physiological differentiation between and within the two types is large enough to explain the geographical and morphological divergence as well as the maintenance of the two types by natural selection. Natural selection is shown to act not only on the adult stage but even more sharply on the egg stage."

The present paper has also been concerned with natural selection acting on the early life history of certain groups of fishes. It has been suggested that the relatively large eggs of these fishes confer certain advantages enabling them more readily to survive within a particular combination of biological and physical conditions. Moore (1949) has reached conclusions of particular relevance to such studies. After admitting that the adaptive significance of differences in the rate of development, egg size and temperature coefficient in *Rana* species cannot yet be properly understood, he states that: "The best evidence that these differences are of adaptive significance is first, that they vary in a regular way with geographic distribution, and secondly, totally unrelated forms frequently exhibit these same characteristics when they occur in similar environments. These factors strongly suggest that these characters have become established through the action of natural selection because they confer an advantage on the organism. They are, therefore, adaptive. This method of equating 'parallelisms' with 'adaptive characters' is probably the most objective means of identifying the latter." Similarly Huxley (1940) and Timofeeff Ressovsky (1940) have emphasized the selective value of various characters which are linked with environmental conditions. Mayr (1942) concludes that ecological rules have a twofold importance for the taxonomist; firstly they assist him in arranging his material, and secondly they demonstrate the intimate correlation between environment and the character of animals—in other words they prove the existence of adaptation.

It need only be added that such concepts are considered to have led to a better appreciation of the adaptive significance of egg and larval size of fishes from coastal polar and oceanic waters, than an attempt to fit the facts to an isolated physical factor such as temperature. Undoubtedly the present account is but an approximation to a balanced appreciation of these problems, but it is hoped that this is due more to present limitations of knowledge than to a wrong approach.

Summary

1. Data on the diameters of the eggs and the lengths of the newly hatched larvae show that coastal fishes from arctic and antarctic waters and deep-sea fishes have a marked tendency to produce relatively few, large yolky eggs. Moreover there is an associated tendency for the larvae of arctic and antarctic fishes to be hatched at comparatively advanced stages.

2. Such trends have already been studied in marine, bottom-living invertebrates from circumpolar and oceanic waters. In particular a high percentage show a suppression of the pelagic phase of development. There is evidence that this may have also occurred in some fish species.

3. In fishes it is suggested that the production of relatively large eggs (hatching into large larvae) is particularly correlated with the sparse concentrations of

planktonic food in oceanic and polar inshore waters. In the latter environment the advantages of a large larva (over a smaller) are the smaller food requirements in relation to size combined with increased powers of swimming, leading to a widening of the range in search for suitable food. Intra-specific competition would also be reduced while extra size may be an important factor in the competition between species, more particularly if they are closely related. Hatching at an advanced stage with a shortening of the period leading to metamorphosis is likely to be advantageous in high arctic and antarctic waters with a short seasonal plankton production besides widening the size range of organisms which can be eaten after the yolk reserves are exhausted.

Finally there is the possibility that an increased supply of yolk leads to increased rates of growth of embryos and larvae, thus counteracting the retarding influence of low temperatures, while the less the surface : volume ratio in the larvae the less will be the tendency to lose metabolic heat to the surrounding water.

In deep-sea fishes the advantages of producing as large a larva as possible are thought to be mainly of importance in increased swimming speed and relatively reduced food requirements.

Literature Cited

Alcock, A. 1899. A descriptive catalogue of the Indian deep-sea fishes in the Indian Museum, collected by R. I. M. S. S. Investigator, Calcutta, pp. iii to 211.

Beebe, W. 1936. The egg-life of a deep-sea eel. Bull. N. Y. Zool. Soc., **39**. 2: 60–66.

Beebe, W., and M. Vander Pyl. 1944. Eastern Pacific expeditions of the New York Zoological Society. XXXIII. Pacific Myctophidae (Fishes) Zoologica. New York, **29**: 59–95.

Breder, C. M., Jr. 1932. On the habits and development of certain Atlantic Synentognathi. Pub. Carnegie Instn. Washington, **28**. 435: 1–35.

Breder, C. M., Jr. 1938. A contribution to the life histories of Atlantic Ocean flying-fishes. Bull. Bingham Oceanogr. Coll., New Haven, Conn., **6** Art. 5: 1–126.

Breder, C. M., Jr. 1943. The eggs of *Bathygobius soporator* (Cuvier and Valenciennes) with a discussion of other non-spherical teleost eggs. Bull. Bingham Oceanogr. Coll., New Haven, Conn., **8** Art. 3: 1–49.

Brown, M. E. 1946. The growth of brown trout (*Salmo trutta* Linn.). I. Factors influencing the growth of trout fry. J. exp. Biol. Cambridge, **22**. 3: 118–129.

Bruun, A. F. 1935. Flying-fishes (Exocoetidae) of the Atlantic. Systematic and biological studies. Dana-Report, No. 6: 1–108.

Burke, V. 1930. Revision of the fishes of the family Liparidae. Bull. U. S. Nat. Mus., 150: 1–204.

Carter, G. S. 1940. A general zoology of the invertebrates. Sidgwick and Jackson, London: 1–509.

Castle, W. E. 1932. Body size and body proportions in relation to growth and natural selection. Science, **76**: 365–366.

Dahl, K. 1912. Nogke opgaver for studiet af fiskens tilvaekst i vore ferskvand. Norsk Fiskeritidende, **31**: 135–151.

Delsman, H. C. 1923. Fish eggs and larvae from the Java Sea. 2. *Chirocentrus dorab* (Forsk) Treubia. Batavia, **3**: 38–46.

Delsman, H. C. 1926. Fish eggs and larvae from the Java Sea. 4. *Dussumieria hasseltii*. Treubia. Batavia, **6**: 297–306.

Delsman, H. C. 1929. Fish eggs and larvae from the Java Sea. 12. The genus *Engraulis*. Treubia. Buitenzorg, **11**: 275–286.

Delsman, H. C. 1930. Fish eggs and larvae from the Java Sea. 14. The genus *Pellona*. 15. On *Chirocentrus hypseloma* and *dorab*. Treubia. Buitenzorg, **12**.

Delsman, H. C. 1932–34. Fish eggs and larvae from the Java Sea. 19. The genus *Setipinna*. 20. The genus *Coilia*. 23. *Clupeoides lile*. 24. A few more Clupea eggs. Treubia. Buitenzorg, **14**.

Ehrenbaum, E. 1936. Naturgeschichte und wirtschaftliche bedeutung der seefische Nordeuropas. Lubbert und Ehrenbaum. Hanb. Seefisch. Nordeuropas. Stuttgart: 1.337.

Gardiner, A. C. 1933. Vertical distribution in *Calanus finmarchicus*. J. Mar. biol. Ass., **18**: 575–610.

Gray, J. 1928. The growth of fish. II. The growth-rate of the embryo of *Salmo fario*. III. The effect of temperature on the development of the eggs of *Salmo fario*. J. exp. Biol. Cambridge, **6**. 2: 110–130.

Hardy, A. C. 1928. The work of the Royal Research Ship "Discovery" in the Dependencies of the Falkland Islands. Geog. J., **72**: 209–234.

Hart, T. J. 1934. On the phytoplankton of the south-west Atlantic and the Bellinghausen Sea, 1929–31. 'Discovery' Rep., **8**: 1–268.

HART, T. J. 1942. Phytoplankton periodicity in Antarctic surface waters. 'Discovery' Rep., **21**: 261–356.

HARVEY, H. W. 1950. On the production of living matter in the sea off Plymouth. J. Mar. biol. Ass., **29**. 1: 97–137.

HEUTS, M. J. 1947. Experimental studies on adaptive evolution in *Gasterosteus aculeatus* L. Evolution, **1**. 1–2: 89–102.

HEYERDAHL, T. 1950. The Kon-Tiki expedition. Allen & Unwin, London.

HILDEBRAND, S. F., AND L. E. CABLE. 1930. Development and life history of fourteen teleostean fishes at Beaufort, N. C. Bull. U. S. Bur. Fish. Washington, **46**: 383–488.

HILDEBRAND, S. F., AND L. E. CABLE. 1938. Further notes on the development and life history of some teleosts at Beaufort, N. C. Bull. U. S. Bur. Fish. Washington, **48**: 505–642.

HJORT, J. 1912. (in Murray and Hjort.) The depths of the ocean. Macmillan, London: 1–821.

HUBBS, C. L., AND E. M. KAMPA. 1946. The early stages (egg, prolarva and juvenile) and the classification of the California flying-fish. Copeia, **4**: 188–218.

HUXLEY, J. S. 1940. The new systematics. Clarendon Press, Oxford.

JESPERSEN, P. 1935. Quantitative investigations on the distribution of macroplankton in different oceanic regions. Dana-Report, No. 7: 1–44.

JESPERSEN, P., AND Å. V. TÅNING. 1926. Mediterraneam Sternoptychidae. Rep. Danish Oceanogr. Exped. Medit., **2** Biology A 12: 1–59.

JOHNSEN, S. 1944. Studies on variation in fish in north European waters. 1. Variation in size. Bergens Mus. Aarb., **3**: 3–129.

LÖNNBERG, A. J. E. 1905. The fishes of the Swedish South Polar Expedition. Wissensch. Ergebn. Schwed. Sudpol. Exped., **5**. 6: 1–69.

LÖNNBERG, A. J. E. 1906. Contributions to the fauna of South Georgia. I. Taxonomic and biological notes on vertebrates. K. Svensk. Vet.-Akad. Handl., **40**. 5: 1–104 (Fishes: 99–100).

MAYR, E. 1942. Systematics and the origin of species. Columbia University Press, New York: x + 334.

MOORE, J. A. 1949. Patterns of evolution in the genus *Rana:* in Genetics, Palaeontology and Evolution. Edited by Jepsen, G. L., Mayr, E., and Simpson, G. G., Princeton: 315–338.

PAPPENHEIM, P. 1912. Die fische der deutschen Sudpolar-Expedition 1901–1903. I. Die fische der Antarktis und Subantarktis. Deutsche Sudpol. Exped., **13**. Zool. 5: 161–182.

RASS, T. S. 1941. Analogous or parallel variations in structure and development of fishes in northern and arctic seas. Jubilee Publications of the Moscow Society of Naturalists 1805–1940: 1–60.

REGAN, C. T. 1916. British Antarctic ("Terra Nova") Expedition 1910. Larval and post-larval fishes. Terra Nova Reports, Zool. 1. 4: 125–156.

RILEY, G. A., H. STOMMEL, AND D. F. BUMPUS. 1949. Quantitative ecology of the plankton of the western North Atlantic. Bull. Bingham oceanogr. Coll., **12** Art. 3: 1–169.

ROULE, L., F. ANGEL, AND R. DESPAX. 1913. Deuxième Expédition Antarctique Française (1908–1910) commandée par le Dr. Jean Charcot. Poissons. Paris: 1–24.

SANZO, L. 1931. Uova, larve e stadi giovanili di Teleostei Fauna e Flora del Golfo di Napoli. Salmonoidei and Stomiatoidei: 21–92.

SANZO, L. 1931a. Uova e primi stadi larvali di *Myctophum gemellari* Cocco (= *Scopelus gemellari* C. & V.). Atti R. Accad. Lincei, **14**. 11: 515–519.

SCHMIDT, J. 1930. *Nessorhamphus,* a new cosmopolitan genus of the oceanic eels. Vidensk. Medd. naturh. Foren. Kjøb., **90**: 371–375.

TÅNING, Å. V. 1918. Mediterranean Scopelidae (*Saurus, Aulopus, Chlorophthalmus* and *Myctophum*). Rep. Danish Oceanogr. Exped. Medit., **2** (Biology) A.7: 1–154.

THORSON, G. 1936. The larval development, growth and metabolism of Arctic marine bottom invertebrates, etc. Medd. Gronland, **100**: 1–155.

THORSON, G. 1950. Reproduction and larval ecology of marine bottom invertebrates. Biol. Rev. Cambridge, **25**. 1: 1–45.

TIMOFEEFF RESSOVSKY, N. W. 1940. Mutations and geographical variation: in the New Systematics, edited by Huxley, J. S. Clarendon Press, Oxford: 73–136.

WAITE, E. R. 1911. British Antarctic Expedition, 1907–1909, under the command of Sir E. H. Shackleton, C.V.O. II. Biology, Antarctic Fishes: 11–16.

WAITE, E. R. 1916. Australasian Antarctic Expedition, 1911–1914, under the leadership of Sir Douglas Mawson, D.Sc., B.E. Fishes. Austral. Antarct. Expedit. Sci. Rep., Ser. C. III. 1.: 1–92.

ZENKEVITCH, L. A. 1949. La structure biologique de l'océan. C. R. XIII Congrès International de Zoologie. Paris: 522–529.

ZEUTHEN, E. 1947. Body size and metabolic rate in the animal kingdom with special regard to the marine micro-fauna. C. R. Trav. Lab. Carlsberg. Ser. Chimique, 26. 3: 17–161.

ON THE ROLE OF INVERSIONS IN WILD POPULATIONS OF *DROSOPHILA PSEUDOOBSCURA* [1]

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INTRODUCTION

The salivary gland chromosomes of *Drosophila* provide an exceptional means to study the role of inversions in a wild population because their frequencies can be established with a high degree of accuracy. This is particularly true of *Drosophila pseudoobscura,* in which species Dobzhansky and Sturtevant (1938), and Dobzhansky have described fifteen or more inversions, and the latter has established their geographical distribution throughout its range from British Columbia to Guatemala (Dobzhansky in Dobzhansky and Epling, 1944).

Certain regularities of occurrence soon became apparent during the course of these investigations. Because some inversions overlap each other, Dobzhansky found it possible to arrange the whole number in two phyletic series and then found that one series was centered in the western United States and the other in Mexico. Second, he found that each inversion type has a definite range. Only one occurs throughout most of the species area and the others are variously restricted and some are apparently quite local. Third, he found that the frequencies of some of the more abundant types vary along regular geographical clines. These clines do not coincide; hence each region is characterized by different frequencies of its principal types.

Other regularities led to the belief that the inversions might be an instrument with which to investigate the evolution of these races. Systematic monthly samples which Dobzhansky made at two ecologically different localities known as Pinon Flat and Keen Camp showed that the relative frequencies of three principal inversions change regularly in the spring of the year at Pinon Flat (and also at another nearby station, Andreas Canyon) but not at Keen Camp, 13 miles distant; and at Keen Camp he found that one inversion increased regularly for eight years (Dobzhansky, 1943, 1947a). Later (1948) he found a different seasonal change in the Sierra Nevada, and an altitudinal cline of frequencies; and still later (1952) in this region, a change in mean yearly frequencies which was associated with differences of weather. These regularities indicate an adaptive relation of some sort between the frequencies of different inversions and the environments in which they are found, as Dobzhansky has shown. The purpose of this paper is to consider evidence which bears on this relation.

CHANGES IN FREQUENCY OF INVERSION TYPES AT KEEN CAMP AND PINON FLAT

The data reported in this paper have been obtained from wild populations in the San Jacinto and Santa Rosa Mountains. These mountain ranges are continuous and for our purpose can be regarded as a topographic unit which separates the moister coastal plain of southern California from the Colorado Desert. They lie about 100–125 miles east of Los Angeles. The first named reaches an elevation of nearly 11,000 feet, the latter about 8000 feet. *Drosophila*

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pseudoobscura occurs more or less continuously, but not uniformly, throughout this area. The differences of climate and weather to which its populations are subjected are typified by the altitudinal and spatial zonation of the vegetation which covers these ranges. Climbing from the coastal plain and foothills to the summits one passes through the semi-desert of coastal sage and chaparral, where rainfall is low and summer temperatures are relatively high, to a moister cooler forest, where snow in winter is an important climatic factor. The lower reaches of this forest are occupied by yellow pine with admixtures of *Pseudotsuga* and oak, and the forest proper by yellow pine, oak, white fir, sugar pine, and incense cedar. Descending the interior slope, one passes more abruptly through forest to chaparral into a pinon pine-juniper woodland intermixed with desert shrubs and thence into the typical shrubby vegetation of the uplands of the Colorado desert. Fluctuations of temperature are greater on the desert side, and total rainfall is substantially less than on the coastal side.

Six inversion types of the III chromosome occur in this area, known as Standard (ST), Arrowhead (AR), Chiricahua (CH), Treeline (TL), Santa Cruz (SC), and Pikes Peak (PP) (Dobzhansky in Dobzhansky and Epling, 1944). They will be referred to hereafter by the symbols indicated. The last named has been found in this region only recently (Epling and Mitchell, D.I.S. 25. 1951).[2] The first three generally comprise 90 per cent or more of most samples and are the principal ones to be considered here. Inversions in the other chromosomes are few and rarely seen, except for a complex of three in the right arm of the X chromosome which is associated with modification of the sex ratio. During 1952 it occurred in about 20 per cent of wild males. Its proportions varied from station to station. Its association with inversion types of the III chromosome of adult males appears to be at random.

The collecting station at Keen Camp is on the margin of a drainage basin known as Hemet Valley which lies on the southeast flank of San Jacinto Peak at an elevation of approximately 4500 feet. The Hemet Reservoir occupies the lowest part of this valley, about a mile from the station. The valley in fringed more or less continuously by a forest of second growth yellow pine and the station itself lies in this forest. The station at Pinon Flat is about 13 miles eastward on the north flank of Santa Rosa Peak at an elevation of 4000 feet. The flat is several miles in diameter and overlooks the Colorado desert. It is covered with pinon-juniper woodland and desert shrubs. The topographic gradient between the stations is fairly even (see Dobzhansky and Epling, 1944, for a more detailed description of both stations).

A record of relative frequencies of the different inversions was begun at these stations by Dobzhansky in 1939, was continued by him until 1946 (Dobzhansky, 1943, 1947a) and by ourselves from 1948 to 1952. The gametic frequencies of ST, AR, and CH for the whole period are summarized in table I. They were obtained as follows. Each wild female of a sample was placed in a bottle with the usual food. When larvae from the eggs she had laid were just ready to pupate, one was dissected and its two salivary gland chromosomes were determined. Thus, two of the gametes in her offspring were ascertained. Gametic frequencies of wild males collected in 1952 are also listed.

We shall consider first the progressive changes in frequency of ST, AR and CH at Keen Camp since 1939, and then the seasonal changes at Pinon Flat.

The mean yearly frequencies listed in table I show that ST increased regularly at Keen Camp from 1939 to 1946 and thereafter fluctuated irregularly and tended to decrease, more or less equally

[2] Olympic, known heretofore in California as far south as Santa Barbara County, Estes Park, known heretofore from the Rocky Mountains and Mexico, and two as yet undescribed simple inversions from ST have been found in 1953.

at the expense of AR and CH. The regular increase suggests a causal relation with some factor or factors of environment, as Dobzhansky has pointed out (1947a). Precipitation is the greatest variable of weather in this region and probably the chief cause of different environments in successive years. It is also doubtless one of the principal factors of the environment of *Drosophila pseudoobscura* because of its probable effect on the substrate on which both larvae and adults feed. So far as known this is supplied by slime fluxes (Carson, 1951) which arise from bacterial fermentation of the inner wood of living trees and shrubs. They, in turn, are dependent in part on the water relations of the host, and hence on available ground water and the saturation deficit of the air. The precise habitat of *Drosophila* in the San Jacinto Mountains is unknown, but we can conjecture reasonably that it is a substrate of a similar sort which bears a similar relation to precipitation. We have

TABLE I.

Inversion type frequencies at Pinon Flat

	% ST	% AR	% CH	N
Apr. 24, 1939	50.8	29.5	13.1	61
May 13	27.9	35.8	30.0	240
June 21	29.9	35.1	30.5	154
Aug. 19	35.9	33.3	25.6	156
Sept. 19	51.1	22.6	23.2	190
Oct. 21	54.6	25.4	16.6	284
	41.7	29.8	23.8	1085
Mch. 3, 29, 1940	44.6	20.2	30.1	386
Apr. 21	34.7	28.4	33.5	176
May 10	28.2	27.2	39.6	202
June 1	24.1	30.0	41.8	170
Sept. 14	34.6	25.0	37.5	104
Nov. 2	37.5	32.5	26.3	80
	35.4	25.5	34.5	1118
Mch. 7, 1941	56.4	10.9	23.6	110
Apr. 19	58.2	20.0	17.3	100
May 10	44.0	28.0	24.0	100
June 15, 28	32.9	32.3	32.3	192
Aug. 5	51.9	21.3	25.9	108
Sept. 6–Oct. 4	56.2	18.8	16.3	80
Nov. 8–Dec. 6	45.0	24.0	24.0	100
	47.0	23.2	24.4	800
Apr. 1942	51.0	21.6	19.6	102
May	48.0	17.0	25.0	100
June	29.8	22.8	40.4	114
July	41.9	21.8	30.6	124
	42.3	20.9	29.3	440
Apr. 1945	32.7	31.3	34.1	352
Mch. 1946	56.5	18.3	18.3	558
June	26.2	23.8	43.8	500
	41.7	20.8	30.3	1058
Nov. 1948	53.4	23.3	23.3	30
Apr. 6, 1949	54.5	16.5	25.5	180
May 26	42.5	28.8	22.7	66
	51.3	19.9	24.8	246
Mch 30, 1950	49.0	21.8	26.2	302
Apr. 16	45.0	27.5	25.0	120
May 11, 14	33.1	25.6	41.3	320
	41.5	24.4	32.5	742
Mch 25, 1951	56.6	23.6	19.5	72
May 7	52.5	20.0	25.8	120
	54.2	21.3	23.4	192
Mch. 31, 1952	50.8	22.9	16.9	118
Apr. 20	59.1	20.4	9.1	88
May 23–25	50.0	19.0	23.8	42
June 19–23	34.0	29.2	21.7	106
July 25–27	55.1	20.9	14.8	196
	50.4	22.7	16.4	550
	Adult males			
Mch. 31, 1952	48.6	16.2	18.9	74
Apr. 20	51.3	20.5	20.5	156
May 23–25	30.0	22.5	32.5	40
June 19–23	26.4	32.8	28.6	140
July 25–27	48.7	17.5	20.2	228
Aug. 29–31 / Sept. 11, 12	57.7	19.2	14.4	104
	45.3	21.4	21.6	742
Total (1952)	47.4	22.0	19.3	1292

TABLE I.—*Continued*

Inversion type frequencies at Keen Camp

	% ST	% AR	% CH	N
Apr. 1939	32.5	30.0	32.5	40
May	32.6	28.5	36.2	298
June	24.8	30.8	41.2	718
July	28.6	29.3	37.3	566
Aug.	29.5	30.5	37.6	210
Sept. and Oct.	26.0	35.7	35.1	154
	27.8	30.5	38.3	1986
Apr. 1940	30.6	22.8	42.7	464
May	26.8	22.1	47.1	526
June	31.9	20.7	43.4	728
July	34.5	24.8	37.4	452
Aug.	37.6	26.4	31.5	178
Sept.	41.2	17.6	32.4	34
	31.5	22.5	41.9	2382
May 1941	29.0	29.4	37.9	248
June	38.3	17.6	39.3	290
July	35.4	27.2	33.5	158
Sept.	38.2	25.0	32.4	68
	34.7	24.1	37.1	764
Apr. 1942	45.1	17.6	29.4	102
May	45.1	14.7	33.3	102
June	28.2	15.4	46.4	110
July	26.0	18.0	52.0	100
	36.0	16.4	40.3	414
Apr. 1945	41.0	22.2	29.2	288
Apr. 1946	52.0	15.5	23.7	400
June	48.0	15.0	32.5	400
	50.0	15.3	28.1	800
July 1948	45.0	16.1	39.0	149
Sept.	46.5	22.1	31.4	86
	45.6	18.3	36.2	235
May 1949	38.1	22.0	38.1	168
July	48.7	18.8	32.5	168
	43.3	20.4	35.4	336
April 16, 1950	32.0	21.5	44.5	200
May 27	26.8	21.7	50.0	138
Aug. 27–30	33.2	26.0	40.8	196
	31.1	23.2	44.5	534
Mch. 25, 1951	44.4	26.2	26.2	88
May 9	46.3	19.8	30.1	162
	45.5	22.0	28.8	250
July 4–6, 1952	30.2	30.2	34.0	212
Aug. 8–11	36.0	26.9	30.1	186
	32.9	28.6	32.2	398
	Adult males			
May 1, 1952	42.9	19.6	19.6	224
June 3	32.9	30.5	29.3	82
July 4–6	32.8	23.3	39.4	180
Aug. 8–11	32.2	29.0	32.3	62
	36.8	23.5	29.0	548
Total (1952)	35.2	25.7	30.3	946

found a few small fluxes on oaks near the Keen Camp Ranger Station, mostly dried and inactive, or harboring only *Illicigaster* larvae, but the larvae of *Drosophila* have never been found in this area. Oaks do not occur at the Keen Camp collecting station.

The variation in precipitation from year to year at Hemet Reservoir near Keen Camp is shown in figure 1. The totals were high from 1939–1942 and diminished thereafter, resulting in a severe drought from 1948–1951. This drought was general throughout the Southwest and had a marked and visible effect on the biota. Dobzhansky (1952) noted it at Mather in the central Sierra Nevada and found that the yearly differences in frequency of ST, AR, and CH were correlated with it. We have therefore compared the trend of frequencies of these inversions at Keen Camp with the trend of precipitation at Hemet Reservoir nearby (fig. 1), in order to ascertain whether they might also have been correlated in the San Jacinto Mountains.

The horizontal line, at 100 on the ordinate scale, expresses both the norm of yearly precipitation for the period indi-

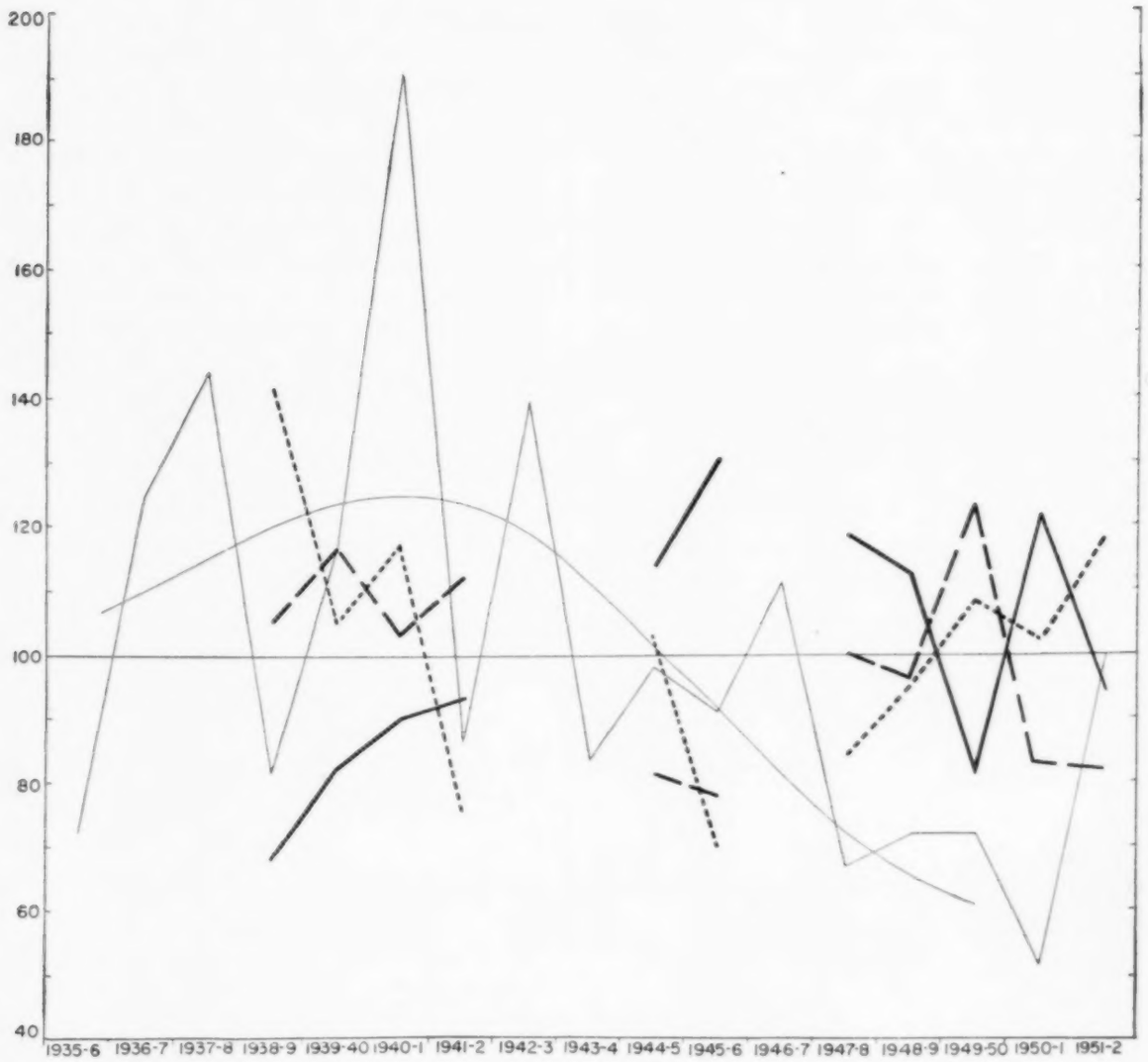

FIG. 1. A comparison of the trend at Keen Camp of mean yearly frequencies of ST (——), AR (····) and CH (– – –) with the trend of precipitation during the period indicated (the smoothed curve). The horizontal line on the scale expresses both the norm of yearly precipitation for the period indicated and the mean of the yearly frequencies of ST, AR and CH.

cated and the mean of the yearly frequencies of ST, AR, and CH. The trend of precipitation thus established is indicated by the smoothed curve; that of the inversion frequencies can be readily estimated by inspection. Thus, one may compare the two trends during this period. The yearly means of precipitation in this figure are not those of total, but of effective precipitation. The latter is an index of the water actually entering the soil and thus available to vegetation and likely to have an effect on the substrate. It excludes interception loss and evaporation loss during and immediately following a storm. The effect of this fraction of precipitation is ephemeral and would be included in the saturation deficit of the air. This index was obtained by converting the recorded precipitation for each storm by means of the equation developed by Rowe and Hendrix (1951) for second growth yellow pine, which is the plant cover at Keen Camp.

Precipitation was above normal during the earlier part of the period. It decreased thereafter and resulted in the drought referred to. ST increased regularly during the period of high precipitation and reached its maximum in 1946 when pre-

cipitation was about normal. Thereafter, it fluctuated from year to year during the drought, when it was decreasing at Mather. ST increased at Mather when the drought was broken in 1951, but decreased at Keen Camp when it was broken there in 1952.

The correspondence between the trends is insufficient to suggest a causal relation between them either by a cumulative effect of precipitation over several years or by differences each year. The latter would be more likely if precipitation influences the substrate of *Drosophila* by its effect on the host, in this case yellow pine; for Rowe and Coleman (1951) have shown that the wilting point of this species is generally reached by early summer because of percolation and evaporation. The effect of precipitation is not therefore cumulative on this host, and one may conjecture that fluxes on it would diminish in summer. The conclusion is accordingly reasonable that the progressive change in frequency of the different inversion types at Keen Camp did not represent a simple response to the trend of weather. The adaptive relation is not so easily discerned. The most perceptible effect of the drought has been on the population density of adults, which had decreased notably from that of the earlier years when we began our collections in 1948, and has continued at a low ebb since, even in 1952, a year of normal rainfall. This decrease has been especially noticed in summer and autumn. Thus, it may be that sustained changes of relative frequency, such as those from 1939 to 1946, occur in this region during periods of high population density and that they are associated in some way with population size.

Seasonal responses, such as those at Pinon Flat, may also be partly a function of population size, and thus only indirectly a response to the yearly rhythm of environments. This rhythm can be estimated at present chiefly by consideration of the principal factors of weather; namely, precipitation, temperature, and relative humidity. We have kept a record of daily fluctuations of temperature and relative humidity at both Keen Camp and Pinon Flat from December 1948 to September 1952, that is, for four precipitation cycles. The instruments used were Bristol monthly recording hygrothermographs. They were housed in standard Weather Bureau instrument shelters kindly loaned for the purpose by the U. S. Forest Service. Records of precipitation at Hemet Reservoir, about a mile from the Keen Camp collecting locality, were generously supplied by the Lake Hemet Water Company. None are available for Pinon Flat. Precipitation at this station follows the same trend as at Keen Camp, inasmuch as most of it is brought by the same storms; but the monthly totals are assuredly much less.

The general trend of weather at Pinon Flat is illustrated in figure 2 if we recall that most of the precipitation falls from October to May. The winter is relatively cold and wet, with infrequent and usually ephemeral snow. Rainfall decreases in spring, as temperatures increase, and the ground dries rapidly because of percolation and evaporation. The hot dry season is prolonged from June to September, broken in some years by showers. Because of lower precipitation and higher temperatures, the summer drought arrives more rapidly here than at Keen Camp, and the change from the conditions of spring and the growing season to those of summer and relative dormancy are much more abrupt.

The seasonal activities of the biota follow this rhythm regularly at each station but differ substantially between the stations. This is illustrated by the increase and decrease of population densities of *Drosophila pseudoobscura*. During winter the maximum daily temperature in this area is frequently below 40° F. and we have observed that growth and activity in cages in the wild are greatly reduced below this temperature. As the season advances, the period each day during which growth and mating may occur are correspondingly prolonged until the cooler

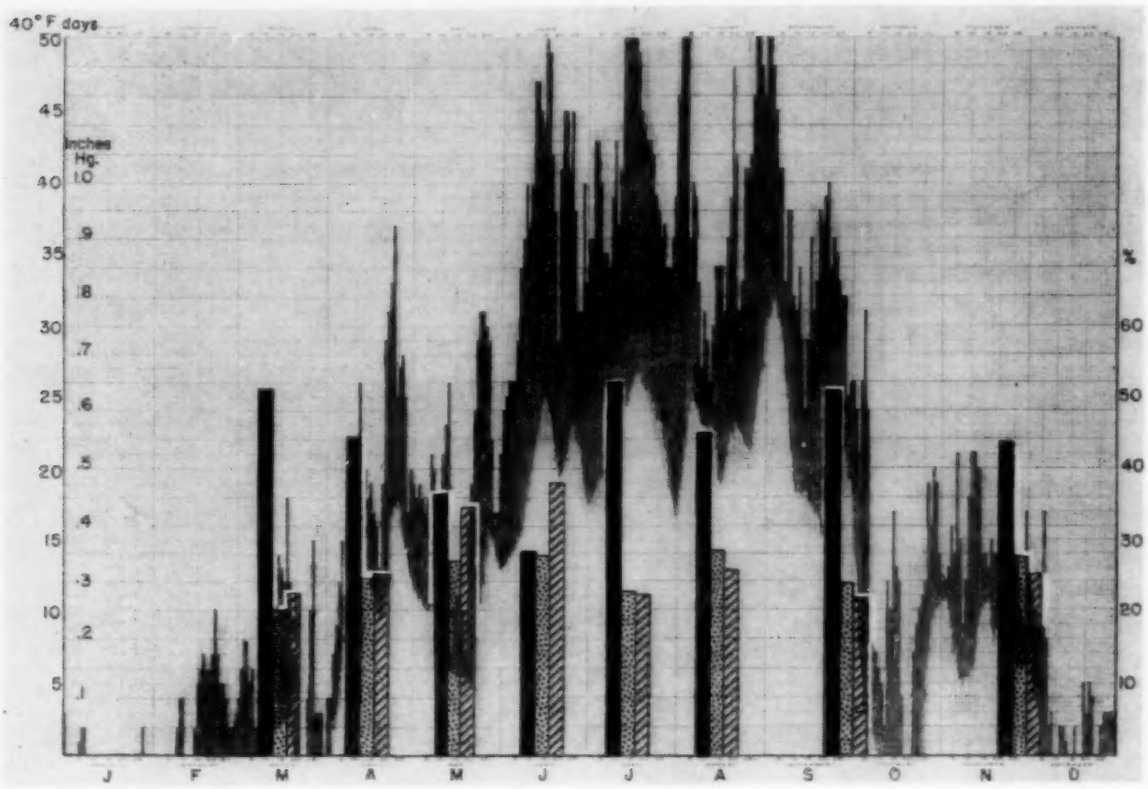

FIG. 2. The mean percentages of monthly frequencies of ST, AR and CH for an eleven-year period at Pinon Flat, shown by the vertical bars in the order named, together with climatic data for 1949 which are explained in the text.

autumn months. We have therefore attempted to illustrate this aspect of the weather cycle instead of a statistic such as mean or maximum temperature. The left hand scale of figure 2 expresses the seasonal increment of heat, that is, the mean excess over 40° F. for each 24-hour period during 1949; and the graph illustrates the seasonal progression of conditions when *Drosophila* populations might be expected to flourish, so far as their thrift is determined by temperature. These conditions are probably curtailed during the warmest months because of high maximum temperatures, particularly at Pinon Flat, but we do not know what these upper limits are. They probably lie between 80°–90° F. The trend of heat increment at Keen Camp is similar. Total heat received there is somewhat less from January to June than at Pinon Flat and notably less during summer and autumn. The saturation deficit, or drying power of the air, is also illustrated in this figure at the lower margin of the shaded area. It follows the temperature trend closely, and is generally reciprocal to the trend of precipitation as might be expected. It is accordingly somewhat less at Keen Camp than at Pinon Flat, specially in summer and autumn. The greatest variations in the trend occur during and shortly after storms.

Observation of cage populations kept out of doors at both localities has shown that reproduction is largely at a standstill at both sites during December and January when temperatures are low. In February and March, when the daily increment of heat above 40° increases, it is renewed, at least by the emergence of adults presumably developing slowly through the winter from eggs laid in November. The population increases in density during April and May, more rapidly at Pinon Flat than at Keen Camp. Adults are abundant at the former and can readily be trapped in late March,

but usually not until late April or May at the latter. This lag in collections at Keen Camp may be in part because of differences of air temperature in the forest with reference to light intensity (Mitchell and Epling, 1951). The maximum is usually reached at Pinon Flat in late May or even in June, and usually in June or July at Keen Camp. From this point the densities differ considerably at the two stations. With the advent of hot days during June and July, the population at Pinon Flat decreases rapidly to a low density, and fluctuates somewhat thereafter, perhaps as a result of summer showers and their effect on eclosion. The decrease is more gradual at Keen Camp and reaches a low density only in late August.

The progression of monthly type frequencies differs markedly at the two stations, as Dobzhansky has pointed out (1943, 1947a). At Keen Camp they fluctuate but do not differ significantly throughout the observable part of the year, regardless of the seasonal changes of environment from April to September and the differences of population density which accompany them. At Pinon Flat, on the other hand, a more regular pattern is followed each year, which is illustrated by the bar diagram in figure 2. Taken at its face value this record of mean monthly frequencies seems to correspond more closely with seasonal fluctuations of population density than with the rhythm of weather, and may be only indirectly associated with the latter. It suggests a regular replacement of ST from March or April to June when the population is large but probably decreasing, followed by a shift from June to July at which time the frequencies of March appear to be reestablished, remaining at about the same level through the hot and dry months of summer and autumn and the cold and wet months from December to April of the following year, when the population densities are low. The means from July to December are based on relatively few samples, however, as will be found by reference to table I. Analyzed more closely (figure 3, from data for the four years during which summer and autumn collections were obtained), the rate of de-

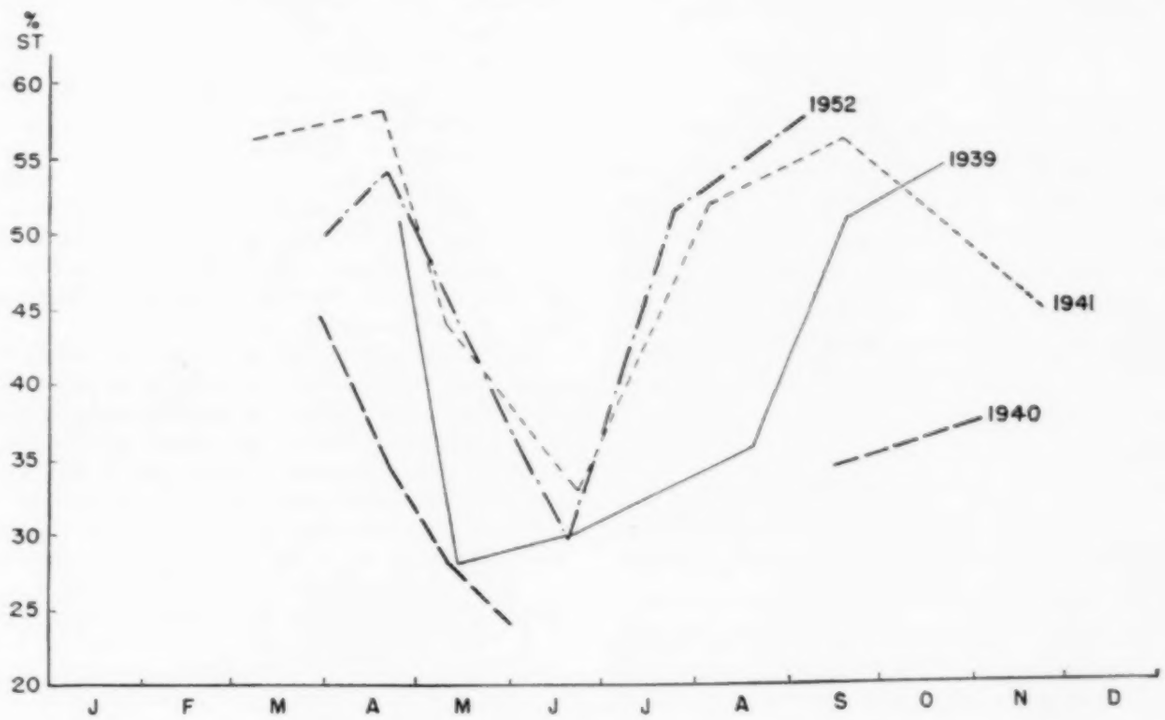

FIG. 3. Rate of change of ST for four years during which summer and autumn collections were obtained.

crease of ST appears to be substantially the same each year, but its rate of increase during summer varies from year to year. It may be gradual, as in 1940, or abrupt as in 1952, when ST regained its preponderance in what may have been a single generation. Scrutiny of weather data suggests no correlation with the differences of weather during these years.

Association of seasonal changes of inversion frequencies both with the seasonal rhythm and with population density appears therefore to be only partial and comparisons, so far as they are possible, suggest that these changes may be substantially determined by factors internal within the population rather than being simple responses of the inversions to the external environment.

Gametic frequencies obtained from the larvae of females are not a sufficient means for evaluating these changes, however, because the responses of the inversion combinations which are actually subject to environmental differences cannot be determined in this way. After reviewing data from laboratory cages we shall accordingly return to an examination of the combinations found in wild males.

Cage Experiments

The regular changes of frequency at Pinon Flat, and the existing clines soon suggested to Dobzhansky that the adaptive character of the inversion types could be tested by cage experiments in which populations were constructed with different proportions of the principal types placed in competition with each other in uniformly managed cage environments. Many such experiments have been reported (for example, Wright and Dobzhansky, 1946; Dobzhansky, 1947a,b,c; 1948b; 1950; Dobzhansky and Levene, 1951; Levitan, 1951).

The cages used by us regularly for similar experiments were of two sorts. The first was a relatively large metal cage constructed of galvanized iron and 50-mesh screen in such a way that pint Kerr jars could be screwed into the bottom and serve as food receptacles. These cages were designed chiefly for experiments out of doors at Keen Camp and Pinon Flat, but were also used for initial experiments in the laboratory. At Pinon Flat and Keen Camp they were kept in standard Weather Bureau instrument shelters, along with a hygrothermograph. We found that artificial populations can be maintained under these conditions only with difficulty. One inhibiting factor was contamination from various sources. Quite large insects can contaminate a cage even through 50-mesh screen. Another factor was high daytime temperature during summer when a whole population might be killed within a few days, particularly at Pinon Flat. This suggests that flies in the wild state have means of retiring to conditions which the cages did not offer, and emphasizes the fact that temperatures and relative humidity of the air, recorded at an arbitrarily chosen point, provide only a general index of the conditions to which the wild flies are actually exposed. Comparisons of such records between stations may accordingly be misleading. This was made particularly clear by a series of records obtained during the summer of 1948 at Keen Camp. A weekly hygrothermograph, contained in one of the large cages referred to, was placed in the crown of an oak tree on the margin of a ravine. A second cage and hygrothermograph was placed within the ravine at the base of an oak tree about 100 feet distant. The differences of mean temperature and relative humidity between the two cages approximated the differences between the means at Keen Camp and Pinon Flat obtained at the same season in the following year.

The second type of cage, in which all the experiments reported here were conducted, was designed by Mitchell. It is a much smaller one of plywood with 50-mesh screen on the sides and so devised that a series of plastic trays, each $2'' \times 2'' \times \frac{1}{2}''$, can be introduced seriatim. The trays in each cage are arranged in three rows of five. Trays with fresh food were introduced at regular intervals and the exhausted trays were removed.

The conditions of food and moisture within a cage doubtless fluctuate as trays are added and withdrawn, but probably maintain about the same average condition throughout the experiment. Since each cage is managed in substantially the same way, one might suppose that this average condition is similar in different cages. The possibility exists, however, that differences of handling in parallel experiments might be responsible for differences in results, because of differences in the food and water relations. We accordingly made a comparison of the metal cage, which employed Kerr jars, and the Dobzhansky cage (1947b; kindly loaned by him), which employed Stender dishes, in order to determine whether obvious differences of cage en-

vironments might affect differently populations made from the same strains. We later made a similar comparison between the Mitchell cage, in which the usual food was employed, and a Fernbach flask in which only liquid food was employed in such a way as to simulate a slime flux. The Fernbach flask was provided with an outlet at the base, so as to avoid accumulation of liquid in it. It was filled with chunks of cork-oak bark and capped with a disc of 50-mesh screen. Another flask was arranged above it so as to deliver a sterilized molasses solution, fortified with certain vitamins, through the screen. The flow was checked by a broad pinching devise on the rubber tubing in such a way as to slowly deliver the liquid drop by drop over the bark. The liquid in the lower flask was inoculated with Fleishmann's yeast and with bits of bark from a slime flux at Mather. The net effect was to produce a thin slimy covering on parts of the bark which resembled the fluxes seen at Mather. The larvae fed on this slime, but the adults seemed to prefer the fresh liquid. The adult population in the flask appeared to be about the same in number as those in the cages. The course of replacement of one inversion type by another was not materially different in the two populations of each experiment. We concluded therefore that the differences or similarities in the results of parallel experiments are primarily the consequence of factors operating within the population itself and not because of differences in management of the cage.

The cages were sampled at approximately monthly intervals by taking fairly mature larvae from each of several trays, putting each portion or a part of it in separate half-pint bottles with fresh food, and allowing all to develop at 15° C. Usually three or sometimes four bottles were prepared from each cage and were more or less equally sampled. Both inversion types in each larva dissected were recorded.

Experimental populations of the first series were prepared from structurally homozygous strains obtained at each of the five stations. Each strain originated in the progeny of a single wild female after a series of brother-sister matings of usually three or four generations. These females were obtained from several collections, made chiefly but not necessarily, during one season. Those from Pinon Flat and Keen Camp were obtained chiefly in 1949 and 1950. Those from the other stations in 1951. It would have been preferable, perhaps, if all could have been obtained from simultaneous collections, but such strains can be got from only a relatively small number of females because of the debilitating effects of inbreeding, and the process of obtaining them is likely to be protracted. The consequence may be that the strains obtained in this way do not represent random samples of the various chromosomes present in a population.

The initial population of each cage consisted of a series of F_1 progenies obtained by crossing two inversion types; hence, the proportions of each type were equal. Usually twelve or more strains of each type were used in making a population and the strains from a given station were mostly the same in all the experiments. Each strain was replicated in making a population. That is, males of a given strain of STST were mated to virgin females from two or three strains of CHCH. The males of the latter were then mated to virgins from two or three other strains of STST. This procedure was carried out in such a way that the progenies of each cross would be available at the same time. Each experimental population was then prepared by mixing approximately the same numbers of flies from each mating. The composition of each cage population and the results of each experiment are presented in table II. The scheme of crosses of the first series of experiments was as follows:

The strains of both CH and ST from Pinon Flat were substantially the same in each experiment, and likewise those from Keen Camp. All of the experiments were conducted at 25° C. The graphs which follow in figure 4 are representative of the results and illustrate those of cages to which we shall refer. The curves are semi-logarithmic, following Levene's method of analysis (Dobzhansky and Levene, 1951) and represent the changing proportions of CH relative to ST. The 95 per cent confidence limits of each sample are indicated by dots.

Reference to figure 4 indicates that three results may follow from competition between ST and CH in what appear to be similar environments. ST may replace CH almost wholly, if not completely (Cage 41); an equilibrium between them may be reached and maintained (Cage 45); or an equilibrium may be reached at substantially the same time and proportions, and then may be lost (Cage 23). In the first instance the ST structural homozygotes retained a greater adaptive value than either the heterozygotes or CH homozygotes; in the second, the structural heterozygotes reached an adaptive value superior to that of either homozygote; and in the third, the adaptive superiority held for a time by them was regained by STST.

Attention is now directed to an experiment of which the results are shown for

convenience by the broken lines on the graph of Cage 45 (fig. 4). Mention has already been made of the parallel experiment in the Fernbach flask, which reached substantially the same equilibrium. When this experiment was discontinued, the adult population of the flask was divided into five portions. Each portion (approximately 250 flies) was put into a bottle with the usual medium and kept at 25°. The adults were transferred at approximately monthly intervals to fresh bottles for approximately 6 generations. Meanwhile, their numbers waxed and waned. The adult populations of the 7th generation of each of two bottles were then transferred to the usual cages and were kept at 25° C. The cages were managed by Miss Joan Malloy. Egg samples were made of this and two subsequent generations. The data follow:

	Generation	1	2	3
Cage I	CH/ST	50/226	53/293	46/254
	0/0	.221	.181	.181
Cage II	CH/ST	31/257	33/245	38/278
	0/0	.121	.135	.136

The differences between the two cages cannot be said not to have been due to sampling error. Nevertheless, although not statistically significant, they were consistently maintained for four generations. To be sure, the sampling error may have been systematic. But this seems unlikely

TABLE II. *Results of 17 cage experiments with the inversion types indicated*

The top row of figures gives the interval in days between the beginning of the experiment and the time each sample was made. The second row gives the per cent of ST in each sample, and the third the sample size.

Cage 22														
PST(M)-PCH(M)	days	42	77	108	138	169	199	227	234	256	282	270	325	360
	%	58	66.5	61.5	72.5	76	86	89	91	91	93	94.5	83	97.5
	N	200	204	210	218	200	252	224	158	240	206	216	180	202
23														
PST×PCH		42	77	108	138	169	199	227	234	256	290	325	360	
		61	76	87	85	84	89	97.5	91.5	94.5	96	97	97.5	
		200	204	220	234	200	254	222	214	224	240	182	204	
26		77	108	138	169	199	227	234	270	290	325			
PST(M)-MCH		54	60	69	77	76	83	83	91	93	92.5			
		224	200	202	204	202	166	230	228	210	200			
28		42	77	108	138	169	199	227	243	263	298	336		
MST-MCH		54	63	73	75	82	80.5	93	93	95	91	96		
		200	182	200	196	214	206	220	204	212	212	208		
29		34	64	103	134	160	189	224	259	295	331			
PST-KCH		57	72.5	79.5	88	81.5	88.5	89	91	95	94.5			
		210	218	204	241	220	232	204	202	208	212			
31		37	66	94	123	164	203	235	256					
MST-PCH(M)		73	71	83	85.5	88.5	91	96	94					
		220	204	244	226	200	250	208	200					
32		37	66	103	123	158	192	228	264					
KST(M)-KCH(M)		66	70	77.5	82.5	87.5	82.5	87.5	90					
		200	218	230	204	218	210	206	200					
33		25	61	91	138	174	209	245						
KCH-KST		64	71.5	88	84	90	94	96						
		212	204	228	236	212	206	208						

TABLE II.—*Continued*

34	25	61	91	124	159	236	289	329			
PCH-KST	65.5	79	79.5	88	83	91	93.5	95.0			
	214	178	208	242	174	214	210	162			
36	38	66	89	122	143	171	205	235			
PST-KAR	40.5	71	75	78	82	79	85	85			
	204	222	208	238	208	204	204	176			
38	35	66	89	122	157	193	222	287			
KST-PAR	66.5	74	74.5	80	76	79.5	79	78			
	208	214	212	232	226	206	200	200			
39	23	51	79	129	150	185	212	249	299	335	404
PCH-TST	47	62	69.5	76	81	82	91	92	95	100	94
	200	216	130	218	208	206	204	200	206	200	210
40	51	79	129	150	185	212	249	335			
PCH-VST	57.5	82	84	86	87	94	91	98			
	204	220	220	220	204	208	206	203			
41	23	54	101	135	175	207	241				
KCH-TST	50	69	73	80	92.5	94.5	97.5				
	202	246	226	212	234	204	212				
42	65	101	135	178	207	341					
SST-PCH	66	80	75.5	92.5	96	96					
	228	254	204	204	204	206					
43	54	90	124	160	174	201	238				
KCH-VST	82	84.5	88	95	93	93.5	94.5				
	210	218	206	104	208	202	204				
45	50	84	128	162	220	256					
SST-KCH	68	70	89	85	86	87					
	202	206	210	200	172	214					

in view of the method. Two interpretations are possible: either that the equilibrium which had been reached in the Fernbach flask was maintained in the bottles and subsequently in the cages for a total of approximately 15 generations, or that a new equilibrium was reached in each series of bottles and was subsequently maintained in the cages to which the two populations were transferred. The latter explanation is consistent with the results of Cage 23.

In all of the experiments referred to above, only the III chromosome was taken into account. In the experiments in which two inversions from the same locality were used, the four other chromosomes of each cell were those with which the III chromosome would normally be associated. In those in which the inversions were from different localities, the other chromosomes would be mixtures from both localities. It seemed desirable therefore to test III chromosomes from the same and from different localities against a background of other chromosomes which would be both homogeneous and different from the usual one. Thus, one might learn to what extent the interactions of the inversions were modified by the remainder of the genome.

The experimental population of the second series was obtained by the following procedure. The first step was to obtain a Mather stock in which the III chromosome should bear the marker genes *or* and *pr*, but with the remainder of the genome intact. This was done by crossing *or pr* males to virgin females of the Mather stock (STST), inbreeding the F_1 by brother-sister matings, selecting homozygous

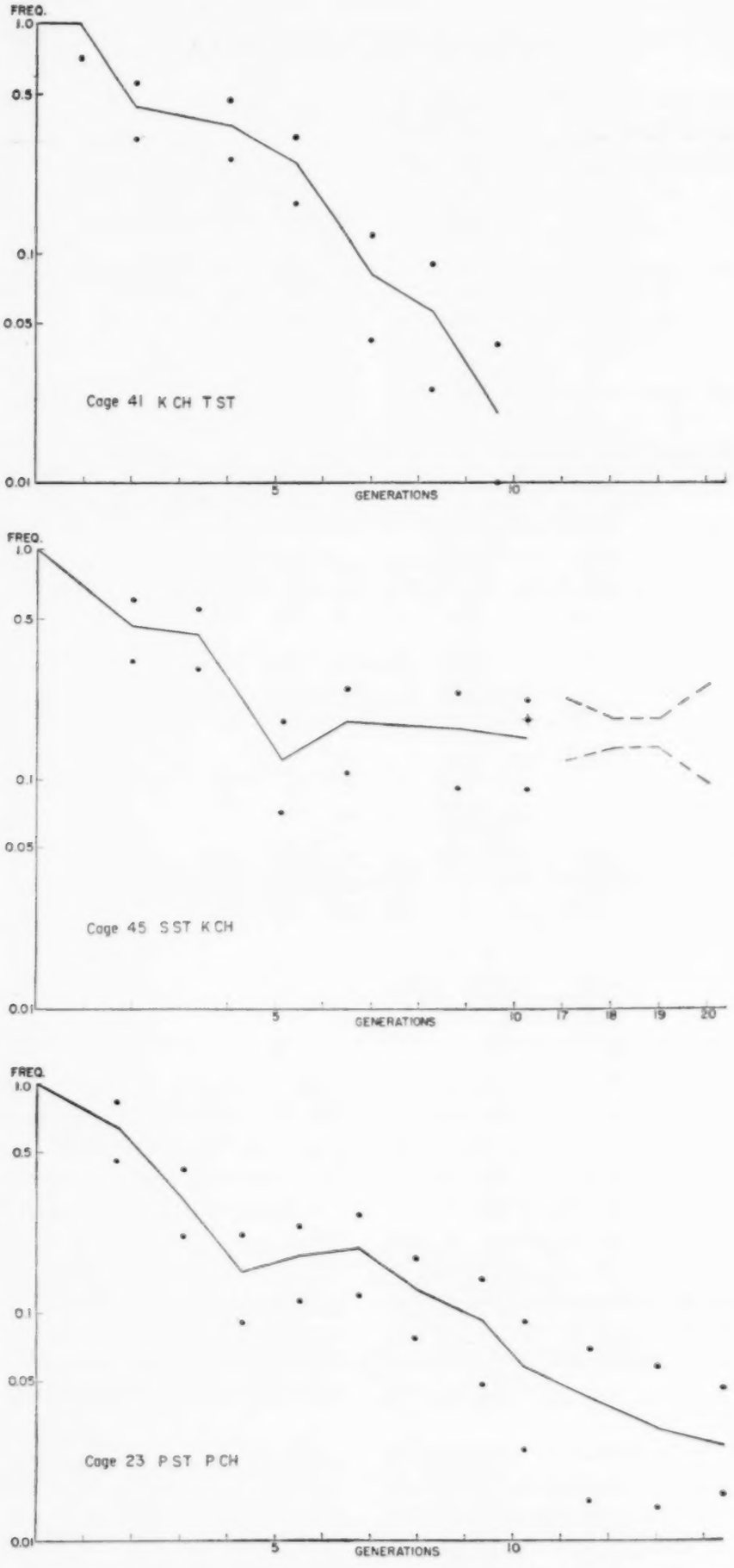

FIG. 4. Results of competition between ST and CH in three cages discussed in the text. Ratio of CH : ST is shown semilogarithmically, with the 95% confidence limits of each sample. The broken lines on the graph of Cage 45 are discussed in the text.

or pr males from their progeny, and backcrossing them to virgins of the original Mather stock. This was repeated eight times. Having this stock, two concurrent series of crosses were then started. One involved transfer of a similar set of markers, *or*, *Bl*, *Sc*, and *pr* to a Mather stock of CHCH, following the same procedure. These markers are balanced over a lethal inversion. *Bl* is lethal when homozygous. In the other series flies from the Mather *or pr* stock already obtained were crossed to twelve strains each of STST and CHCH from Pinon Flat, and STST and CHCH from Keen Camp. The F_1 males from these progenies were then backcrossed to virgin Mather *or pr* females, and the resultant heterozygous males were backcrossed again. This was repeated eight times. Thus, two stocks were at hand in which the II, IV, V, and X chromosomes were from Mather and in one of which some of the III chromosomes were from Pinon Flat or Keen Camp. These were now crossed by mating males in which the III chromosome was heterozygous for *or pr* and wild type from Pinon Flat or Keen Camp, to females heterozygous for *or Bl Sc pr* and CH from Mather. Flies heterozygous for *or Bl Sc pr* and wild III chromosomes from Pinon Flat or Keen Camp were chosen from the progenies and inbred. A final selection was then made for flies carrying III chromosomes for STST or CHCH from both San Jacinto stations which were thus combined with the remaining chromosomes from Mather. Eight strains of each type were ultimately obtained and combined in the cages in the way described for the first series.

The results were similar in all these experiments. ST replaced CH (table II), but at a slower rate than in those with the San Jacinto backgrounds. Whether this difference can properly be ascribed to the influence of the Mather chromosomes is uncertain. The most striking result of the whole series, however, is its similarity to the other series, which suggests that polygenic systems represented in both localities are similar in their capacity to adapt a cage population to the conditions of the cage. This interpretation can also be made of the results of an experiment reported by Dobzhansky and Levene (1951) in which CH from Santa Barbara, Chihuahua reached an equilibrium with ST from Pinon Flat similar to that of Cage 45 in which the types were drawn from populations much closer to each other and occupied similar habitats.

These results suggest that the coadaptive relation of two types in competition are dependent in part on internal differences which may accrue during the course of the experiment and that the fitness of a population may be only indirectly dependent on the relative frequencies of the inversion types present.

Thus, the combined data from our cage experiments indicate that an equilibrium reached between two competing inversion types is a consequence of polygenic recombination within and between each chromosome type, as well as others of the karyotype. Dobzhansky (1949, 1950) has reached similar conclusions though, to be sure, in a somewhat different way. It would also seem that a coadaptedness which exists between two inversion types may be transitory and a function of the polygenic complexes formed at any given time, and not necessarily a characteristic of the inversions present except so far as they may canalize recombination by suppression of crossing over (Dobzhansky and Epling, 1948).

Changes in Frequency of Structural Homozygotes and Heterozygotes

The results of the cage experiments reported above suggest that the relative frequencies of two inversion types in competition are determined by recombinations within each type, conditioned by the inversion linkages of each. Observation of the relative frequencies of inversion homozygotes and heterozygotes in wild populations confirms this view. These observations were made by determining the two inversion types which had been combined at fertilization in each wild male in samples collected systematically during 1952 at five stations in the San Jacinto Mountains, including Pinon Flat and Keen Camp (table III).

The five stations referred to had been carefully chosen, after a conversation with Dr. Bruce Wallace, so as to give a comparison of stations which were similar or dissimilar ecologically and arranged in

TABLE III. *Observed zygotic frequencies of combination classes in adult males*

	Keen Camp				Thomas Mountain				Vandeventer Flat			
	5/1	6/3	7/4–6	8/8–11	5/1	6/3	7/7,8	8/12–14	4/20	5/23	6/24	7/28
STST	15	3	10	4	11	6	16	6	10	27	6	29
STAR	26	10	16	6	6	9	13	9	21	24	9	18
STCH	26	7	19	6	17	8	17	17	20	25	4	11
STTL	6	2	1		9	3	5		4	6	1	1
STPP	8	2	3		7	4	6	3	4	6	1	2
ARAR	1	4	3	2		3	5	4	2	9	3	3
ARCH	4	5	18	6	12	15	16	13	8	27	6	7
ARTL	3	1	1	1		2	6	1	1			1
ARPP	9	1	1	1	5		3		4	5	3	2
CHCH	2	6	16	3	3	11	24	4	4	13		1
CHTL	7		2	1	4	3	1		2	1		
CHPP	3			1	4	2	3		3	6	1	
TLTL	1				1							
TLPP	1						1		1		1	
PPPP					2	1				1		
N	112	41	90	31	81	67	116	57	84	150	35	75

	Pinon Flat						Santa Rosa Mountain			
	3/31	4/20	5/23	6/19–23	7/25–27	8/29–31 9/11–12	5/1	6/3	7/12–13	8/15–16
STST	10	18	3	5	33	20	4	2	12	8
STAR	6	20	2	17	16	12	6	3	17	11
STCH	5	17	3	2	13	6	10	13	16	6
STTL	1	6	1	2	8		4	2	1	2
STPP	4	1		6	8	2	5	1	2	1
ARAR		2		4	4	1	2	1	9	6
ARCH	5	6	5	17	14	3	11	1	21	5
ARTL	1		1	4	2		4		2	1
ARPP		2	1			3	2		2	2
CHCH	2	4	1	9	5	3		3	10	3
CHTL			3		5		2		5	1
CHPP		1		3	4		4	2	3	2
TLTL		1		1		2				1
TLPP	2				2			1		
PPPP	1									
N	37	78	20	70	114	52	54	29	100	49

such a way that similar and dissimilar stations would be equidistant. Thus, we expected to learn something of the relative effects of distance, vegetation, and altitude on the occurrence of inversion types.

We shall now briefly contrast the stations. Four of them, 5–6 miles apart, are on a line which passes through the Hemet Valley from Keen Camp to Pinon Flat, with about 500 feet difference in elevation. The vegetation changes rather abruptly along this transect, in accord with the climatic gradient to which we have referred: from second growth almost pure yellow

pine to chaparral, then to pinon-juniper woodland and desert shrubs, because of the screening of rainfall by the coastal range. The first two stations, at Keen Camp and Thomas Mountain, are almost identical, the chief difference being scattered specimens of a large bush oak, *Quercus dumosa,* at the latter. The third station, at Vandeventer Flat, is surrounded by chaparral, but is located in a restricted grove of large, ancient and closely spaced live oaks, *Quercus agrifolia.* This is a coastal species which is existing here at the extreme margin of its range, probably because of a small stream. The fourth station, at Pinon Flat, lies in the pinon-juniper belt which forms the uppermost zone of desert vegetation. The fifth station, on Santa Rosa Mountain, is roughly equidistant from both Pinon Flat and Vandeventer Flat, and thus forms an equilateral triangle with them. It is about 2000 feet higher and is situated in a yellow pine forest which includes white fir, incense cedar, and oaks, probably because of lower mean temperatures and a higher rainfall and snowfall. This forest resembles that of the mid-Sierra.

Thus, two stations, Keen Camp and Thomas Mountain, are very like each other; a third, Santa Rosa Mountain, is similar to both, but the vegetation is more diverse and the temperature and rainfall regimes are different. The stations at Vandeventer Flat and Pinon Flat are unlike any of the others, and particularly that on Santa Rosa Mountain, which is nevertheless equidistant from both along a similar topographic gradient.

The zygotic combinations were determined by examination of offspring obtained by mating each male to a virgin of STST stock. The inversions in the glands of four larvae from each mating were then determined. If all were of the same combination class (such as STCH or STST) four more larvae were examined. If all were still of the same class the assumption was made that the male parent was homozygous, hence, the probability of having missed a heterozygote would be 1 in 128. The frequencies found are reported in table III.

Should a given class of inversion types have an adaptive advantage or disadvantage in nature, its frequency would be expected to deviate from that required by the Hardy-Weinberg rule of random association. The probability of such deviations was tested in our samples by first

TABLE IV. *Results of a statistical analysis of deviations of male homozygotes from the numbers expected from the Hardy-Weinberg rule in samples made in the San Jacinto Mountains during the collecting season of 1952*

Sample size and P values, using only one tail of the normal curve, are given. The plus and negative signs in parentheses indicate a corresponding excess or deficiency of homozygotes.

	Keen Camp		Thomas Mt.		Vandeventer Flat		Pinon Flat		Santa Rosa Mt.	
	N	P	N	P	N	P	N	P	N	P
March							37	(+).25		
April					84	(−).02	78	(−).18		
May	112	(−).*002*	81	(−).15	150	(+).14	20	(−).29	54	(−).015
June	41	(+).33	67	(+).28	35	(−).30	70	(+).47	29	(−).09
July	90	(+).48	116	(+).*005*	75	(+).42	114	(+).20	100	(+).33
August	31	(+).49	57	(−).13			52	(+).02	49	(+).08
	Total individuals = 1542						Total samples = 22			

	plus	minus	
not significant	11	6	$h^*-H=-2.4142$
possibly significant	1	2	$\Sigma\sigma^2=310.69$
significant	1	1	$t=-.137$
			$P=.44$

applying to them a method devised by Levene (Dobzhansky and Levene, 1948) for testing the observed frequencies of only one class, namely, homozygotes, particularly in small samples. The results are set forth in table IV. They show that the observed frequencies of homozygotes deviated from the expected in both directions and that these deviations were sufficient to suggest a possible adaptive response in only two samples, namely, those at Keen Camp in May and at Thomas Mountain in July (or at most in five samples). In the first instance they were deficient, in the second in excess. Nevertheless, they might still have a systematic advantage in the region, which would not be disclosed by inspection of this sort. The 22 samples were accordingly combined, following Levene's procedure, with

TABLE V. *An example of χ^2 procedure, employing the samples obtained at Keen Camp on May 5, 1952, and at Thomas Mountain on July 7–8, 1952*

The brackets indicate groupings of low frequencies which would probably distort the results if considered separately. The same grouping was consistently followed in each sample. Five degrees of freedom are assumed.

	Keen Camp				Thomas Mountain			
	Obs.	Exp.	*d*	d^2/e	Obs.	Exp.	*d*	d^2/e
STST	15	20.57	5.57	1.508	16	11.48	4.52	1.780
STAR	26	18.85	7.15	2.711	13	15.10	2.10	2.920
STCH	26	18.85	7.15	2.711	17	26.74	9.74	3.548
STTL	6	8.14	2.14	.563	5	4.09	.91	.202
STPP	8	9.00	1.00	.111	6	4.09	1.91	.892
ARAR	1	4.32	3.32	2.551	5	4.97	.03	—
ARCH	4	8.64	4.64	2.491	16	17.59	1.59	.144
ARTL	3	3.73	4.14	2.181	6	2.69	3.62	2.435
ARPP	9	4.13			3	2.69		
CHCH	2	4.32	2.32	1.246	24	15.57	8.43	4.564
CHTL	7	3.73			1	4.76		
CHPP	3	4.13			3	4.76		
TLTL	1	.81	.57	.028	0	.36	5.97	3.249
TLPP	1	1.78			1	.73		
PPPP	0	.98			0	.36		
Total	112	111.98			116	115.98		
				16.101				19.734

P = .01		P = .002	
ST — 96	42.8%	ST — 73	31.5%
AR— 44	19.6	AR— 48	20.7
CH— 44	19.6	CH— 85	36.6
TL— 19	8.5	TL— 13	5.6
PP— 21	9.4	PP— 13	5.6
224		232	

Levene test:

$\Sigma\sigma^2 =$	18.806
$t =$	−12.37
$P =$	.002

$\Sigma\sigma^2 =$	21.688
$t =$	+2.59
$P =$	.005

TABLE VI. *Seasonal variation all stations, 1952*

	March through May			June through August		
	Obs.	Exp.	d^2/e	Obs.	Exp.	d^2/e
STST	98	102.27	.178	160	132.29	*5.804*
STAR	111	105.53	.284	166	172.73	.262
STCH	123	121.83	.011	145	199.19	*14.742*
STTL	37	30.56	1.357	28	30.61	.223
STPP	35	39.52	.517	41	32.88	2.005
	(40.7)			(37.8)		
ARAR	16	27.22	*4.625*	52	56.38	.340
ARCH	78	62.86	3.646	147	130.04	2.212
ARTL	10	15.77	2.111	22	19.98	.204
ARPP	28	20.39	2.840	18	21.46	.558
	(21.0)			(24.7)		
CHCH	29	36.28	1.461	98	74.98	*7.067*
CHTL	19	18.20	.035	18	23.04	1.102
CHPP	21	23.54	.274	21	24.75	.568
	(24.3)			(28.4)		
TLTL	2	2.28	.034	4	1.77	2.810
TLPP	5	5.91	.140	5	3.80	.379
	(6.1)			(4.3)		
PPPP	4	3.82	.008	1	2.04	.530
	(7.9)			(4.7)		
Total	616	615.98	17.521	926	925.94	38.806
P			.06			*<.001*

Levene test:

$\Sigma\sigma^2 =$	109.3796	201.3796
$h^* - H -$	28.5021	+ 26.0876
$t -$	2.73	+ 1.84
P	*.003*	.025

The gametic frequencies of each inversion type are shown in parentheses.
Ten degrees of freedom are assumed for each sample.

the result (table IV) that the combined t value was negative (− .137) thus suggesting a systematic deficiency in this area during 1952, even though the P value (.44 for one tail of the normal curve) was in no way significant. The lack of significance is in agreement with Levitan's (1951) observations on *D. robusta*. But should our data be combined with those of Dobzhansky and Levene (1948) on the 66 widely distributed samples they analyzed, which were collected during several years (or even with those from the same area), a real deficiency of structural homozygotes and, thus, a superior adaptive advantage of heterozygotes, would be indicated. Our results would, however, substantially reduce the P value of the total evidence.

So far as this analysis is concerned, the data seem to be definitive. The fact remains, however, that useful information may be lost in the attempt to generalize from combinations of data. We have accordingly also analyzed our samples by computing the gametic frequencies of each type in each sample, have used the values thus obtained for expanding the Hardy-Weinberg formula, and have thus arrived at the expected frequencies of each combination class (such as STST or STAR) if the inversion types should be randomly associated in the adult as they appear to be in the egg (Dobzhansky, 1947a, 1948; Dobzhansky and Wright, 1946). Thereupon the differences between the expected and observed frequencies of each class were tested by χ^2, and the χ^2 value for

the whole sample was computed. Several examples are shown in tables IV–VII, as being the best way to illustrate the diverse and complex changes which occur in different samples at different times. A significant value for the total χ^2 indicates that some or all of the different inversions present departed from random association. In some samples a single class may have been the chief contributor to this value, and itself significantly deviant as for example ARPP in the April sample at Pinon Flat (table VI). In other samples, no single class may have been significantly deviant, and a principal contributor to the total. Or, as in the February sample (table VI), the total χ^2 may not have been significant, but one class (ARAR) may have been. Thus, we may get more detailed information of the changes occurring in successive samples. Because of the small numbers involved in the less frequent classes the latter were consistently grouped in the separate samples for 1952, as illustrated in table V, but not in the combined samples nor in the Pinon Flat samples for 1953. Five degrees of freedom were assumed in the first instance and 10 in the second.

Turning now to table V and considering the frequencies of structural homozygotes as a class, we find that the Levene test discloses a significant deficiency in the first sample and an excess in the second. No single homozygote was sufficiently deviant as to be significant for approximately 1 degree of freedom. In order to ascertain whether systematic station to station or seasonal differences might exist, the samples were then variously grouped. When the Levene test was applied to a grouping by spring and summer months

TABLE VII. *Seasonal variation, Pinon Flat, 1953*

	Feb. 2, 3		March 10		Apr. 23		May 10	
	Obs.	d^2/e	Obs.	d^2/e	Obs.	d^2/e	Obs.	d^2/e
STST	80−	.088	73−	.031	78+	1.445	37+	1.195
STAR	59+	.977	48+	.012	65+	.001	23−	1.936
STCH	20+	.496	14+	.292	28−	3.156	16−	1.520
STTL	21−	.006	22−	.008	14−	.357	15+	.780
STPP	11−	1.184	16+	.047	19−	1.697	17−	.347
	(61.0)		(60.5)		(48.3)		(42.6)	
ARAR	2−	4.631	5−	.828	5−	6.996	5−	.901
ARCH	5−	.060	3−	.184	23+	1.046	15+	1.633
ARTL	10+	1.605	10+	1.175	11+	1.313	4−	.414
ARPP	7+	1.020	7+	1.008	25+	13.558	20+	10.808
	(19.1)		(19.2)		(22.9)		(21.1)	
CHCH	—	.880	—	.490	9+	2.033	6+	1.229
CHTL	1−	.662	2+	.018	6+	.347	2−	.926
CHPP	2+	.112	1−	.043	6−	.248	6−	.117
	(6.3)		(4.9)		(13.8)		(15.0)	
TLTL	—	1.380	1−	.282	—	.990	1+	—
TLPP	3+	.539	1−	.727	3−	.002	3−	.077
	(7.9)		(9.1)		(5.8)		(7.6)	
PPPP	1+	1.286	—	.770	—	2.400	—	3.110
	(5.6)		(6.2)		(9.1)		(13.5)	
Total	222	14.946	203	5.915	292	35.590	170	24.993
P		.13		80		.001		.005
Σ^2		31.953		27.232		47.749		28.233
h*−H		−11.60		− 6.652		− 2.019		+ 1.937
t		− 2.05		− 1.27		− .292		+ .363
P		.02		.10		.30		.36

The gametic frequencies of each inversion type are shown in parentheses.
Ten degrees of freedom are assumed for each sample.

the homozygotes were found to be significantly deficient in the samples collected from March to May and in excess from June to August, but not significantly so in the latter if one takes a P value of .01 as the criterion (table VI). Thus, it would appear that the relative frequencies of homozygotes and heterozygotes conform during summer to those expected of random association, and that the former are deficient in spring, indicating an adaptive disadvantage during this period. The samples collected thus far in 1953 at Pinon Flat, Vandeventer Flat, Thomas Mountain and Keen Camp conform to this pattern and those from the first named indicate that a seasonal trend may be occurring (table VII). A marked deficiency of homozygotes occurred there in February, was less in March, slight in April, and passed to an excess in May.

Turning again to table VI we note that the deficiency of homozygotes during spring was contributed chiefly by ARAR just as at Pinon Flat in 1953, and that this homozygote seems to be generally deficient. We also note that both STST and CHCH were significantly in excess in the combined summer samples and that they alone account for 82% of the homozygotes. Three other observations are of interest. First, the excess of STST and CHCH was largely compensated for in 1952 by a strong deficiency of STCH. Second, this relation between a homozygote and one of its heterozygous combinations may change from month to month: ARPP was strongly in excess at Pinon Flat during April and May but the deficiency of ARAR dropped substantially. And, finally, that the percentage frequencies of AR inversions at Pinon Flat did not change appreciably during a period when the marked changes in its combination classes, which are illustrated in table VII, were taking place.

Thus, it would appear that an adaptive disadvantage is not necessarily a property of inversion homozygotes, nor an advantage of heterozygotes, as classes, and that different types of both may differ in their seasonal responses. Whether ARAR is regularly at a disadvantage in the San Jacinto Mountains remains to be seen, but the high frequency of AR in some localities of the Great Basin (Dobzhansky and Epling, 1944) suggests that it may have an advantage there and, thus, that different inversion homozygotes may respond differently in different regions.

The results of this analysis are therefore concordant with our observations on gametic frequencies from wild females and with those of our cage experiments. They suggest that differences in relative frequencies of inversion types are determined by polygenic recombinations which occur within and between them, and that such differences are only partial and inadequate indices of the fitness of the populations they characterize, reflecting in a gross way subtle and as yet unspecified changes in an interacting polygenic system.

Discussion

Drosophila pseudoobscura ranges continuously from the lowlands of British Columbia and Washington through the stark deserts of the southwestern United States to the temperate highlands of Guatemala. It persists in this range in countless habitats and through a series of environmental rhythms, both physical and biotic, as different as those of the redwood border forest of northern California, the pine woodland and desert shrubs of Pinon Flat, and the cypress groves of Guatemala. How then, one might ask, does each of its populations persist in the environment it occupies, subject to great seasonal differences from generation to generation, and how may the species have come to occupy such a diversity of habitats?

Evidence is accumulating that a species of *Drosophila* may be equivalent in its potentials of polygenic combination throughout its range. Dubinin (1948[3]) has

[3] Ozalid copies of an English translation of this paper, made by Prof. I. M. Lerner, can be obtained from the Librarian, University of California, Los Angeles 24.

shown that strains of *D. melanogaster* from localities as widely separated and as ecologically different as Moscow and Alma Ata (in central Asia on the border of Sinkiang), as well as others, are possessed of the capacity to form the complex morphological trait *network* after inbreeding and selection. Goldschmidt, Hannah and Piternick (1951) have stressed the ubiquity in laboratory stocks of factors of diverse origins which lead to the *podoptera* trait when they are combined in a multiple-factor system. A similar possibility for adaptive traits in *D. pseudoobscura* is suggested by the results reported by Dobzhansky and Levene (1951) of competition between ST and CH from California and Chihuahua, and by our Mather background experiments. This body of evidence suggests to us that different parts of *D. pseudoobscura* may have access to polygenic elements from which adaptive systems can be formed by recombination which are similar in reaction to those which can be formed by strains from the same locality.

The variable adaptedness of individuals which might ensue from wide polygenic recombination within the species would be likely to result in relatively few of the progeny of a localized group being adapted to the limited environments to which it would be confined by its very nature. Agents which canalize recombination within each local environment would accordingly tend to perpetuate the recombinants adapted to it and would thus tend to conserve the fitness of each local population. The adaptability of the whole species would also be fostered.

Numerous studies have illustrated the very local control of environment on the prevailing biota, and observation of populations of *Drosophila* in California and experiments on them indicate that *D. pseudoobscura* is no exception. Mattson and Lönnemark (1939), for example, have illustrated the complex character of Swedish soils and subsoils which may exist over a distance of only a few feet, indicating at the same time the association of different types of surface vegetation. Working on the Colorado desert, Shreve (1931) has shown a very local effect of the thin shade cast by *Parkinsonia aculeata* on air and soil temperatures, and thus on the germination and successful establishment of seedlings beneath its crown. The well-known commensalism of many desert plants doubtless arises from similar causes. Precipitation can vary as much as 20 per cent within an acre (Musson, 1902; Humphrey, 1933); and different types, even of herbaceous vegetation, can intercept different amounts (Clark, 1940). Thus, very local differences of ground cover, litter, soil type, the slope and its exposure to sun and prevailing winds, may alter the very local distribution of biota (Potzger, 1939).

Even flying species may be segregated into sedentary local populations by what seem to be minor ecological and spatial barriers, as recently pointed out by Dowdeswell, Fisher and Ford (1949); and Dobzhansky and Wright (1947) have shown that ten months after the release of marked flies at Mather about 95 per cent of their progeny were found within a radius of approximately one mile from the point of release. The dispersion of native flies may actually be less inasmuch as the marked flies, which were bred from laboratory stocks, were released from mid-June to mid-August, when the normal population was probably decreasing, and the habitat was saturated. This being true, one might expect a wider dispersion of the introduced flies because of adult competition. Dobzhansky and Wright have also noted the possibility of differences in local density of a population and the authors of this paper recall a particular shady grove at Mather, visited with Prof. H. L. Carson, where the incidence of slime fluxes was higher than in most of the surrounding area and *Drosophila* was correspondingly abundant. The probability of local differences in density is impressed on one even more fully by repeated years of collecting in the same area. The character and distribution of the sub-

strate may therefore tend to restrict recombination locally. Mitchell and Epling (1951) have also suggested that differences in density of vegetation may extend or curtail the daily activities of adults because of their reaction to light and thus may lead to differences of recombination within a locality. Individual physiological reactions may also differentiate an already spatially limited population along the course of time. Thus, Epling and Lewis (1952) have described a condition in *Delphinium* which seems to involve a differential response to weather in respect of the water relation. All of the individuals of a colony do not necessarily flower or set fruit each year. Different individuals do so each year, apparently as a result of weather during the growing and flowering seasons. Other similar agents which localize recombination have been discussed recently by Baker (1951). They are but little studied in *Drosophila*.

The fitness of a local population to its environment may accordingly be perpetuated in part by the territoriality of its components and the ecological control of migrants from other localities, resulting in a restriction on recombination and canalyzing it in accord with the properties of the habitat. Its fitness would be further enhanced by the interrelations of hybrid chromosome structures such as inversions, translocations and localization of chiasmata which induce differences of linkage and further restrict and canalize recombination. These would also contribute to the adaptability of the whole population as Dobzhansky has recently pointed out (1951). An imperfect analogy will illustrate how this may be. Let us liken a local population such as that at Pinon Flat to a balance with fifteen pans instead of two. Each pan will correspond to an inversion combination of the III chromosomes. Genetic systems which involve genes linked within each combination will correspond to the weights to be put in each pan. The population, like the balance, is in equilibrium whatever the respective positions of its pans. But their positions will be determined by the frequencies of the adapted genotypes in each, that is, by the weights in the pans and not by the pans themselves.

A pair of inversions may change its coadaptive relation within a population, just as within a cage. Thus, ST and CH were differently coadapted in the spring samples than they were in the summer samples, for the differences subsumed in the combined samples were more or less systematic in each separate sample. Other pairs may change very little, as seems to have been consistently true of STAR. Should changes in coadaptedness result in the increase of a given combination, then the position of its pan would change correspondingly, just as ARPP increased during April and May at Pinon Flat. But the relative frequencies of its component inversions would depend on changes in other combination classes as well. For the population, like the balance, is responding as an integrated whole.

Differences of interaction between the linkages of inversion types and other chromosomes may be enhanced by another effect of an inversion noted by Levene and Dickinson (1952) which indicates that different inversion heterozygotes in the III chromosome increase recombination in the X chromosome differentially. This being so, different frequencies of inversion types in local populations would still further modify their patterns of recombination and one would accordingly expect the positive correlation of chromosomal polymorphism with diversity of habitats which da Cunha, Burla and Dobzhansky have reported for *D. willistoni* (1952).

The fitness of each localized population to its effective environment and the factors which preserve it during successive generations are functions, not of types such as inversions, but of the coadapted polygenic systems which are combined in each genotype. Fitness is, thus, a function of individual adaptedness; which was Darwin's thesis. The adaptedness of individuals in a locality, however, is secured and perpetuated through the medium of

an integrated genetic system within each breeding population, its "gene pool," as Dobzhansky (1949) and Wallace (1952) have so clearly shown. The individual and the breeding group to which it contributes are accordingly the effective vehicles of evolution in sexually reproducing organisms (Epling and Catlin, 1950). Each conditions the other, and the interplay between them determines the "breeding system" of the group. Inversions, when present, are factors of these coadapted systems and provide what may be a substantial condition for preserving both individual adaptedness and the fitness of the population, both locally and in all its parts. This, we suggest, may be their principal role.

The study of inversion types in *D. pseudoobscura,* particularly by Dobzhansky, has made important contributions toward an understanding of this dual relationship. Further understanding depends on methods which will apprehend the factors that lead to coadaptedness of two systems within an individual and their relation both to its survival to sexual maturity and its subsequent reproductive adaptedness to other individuals. Wallace (1952) and Wallace and King (1952) have made a discerning start in this direction.

Summary

Data are presented from this standpoint: Are structural heterozygotes which result from different inversions in the III chromosome of *D. pseudoobscura* adaptively superior to the corresponding homozygotes? The evidence indicates that this is not the case. The coadaptive relation between types may differ in different wild populations and at different seasons, and similar differences may occur in cage populations. These differences need not be equal for different pairs of inversions. The fitness of the carriers of both homozygous and heterozygous combinations is variable and may be transitory. The differences are interpreted as a consequence of polygenic recombination, both within the inversions and between them and the other chromosomes present. Fitness is a consequence, therefore, of recombinations constituting a whole genotype. The inference is drawn that by their presence inversions may assist in canalizing the fitness of a local population to its particular milieu by reason of their restriction on recombination and that they may thus conserve the adaptability of the species.

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References

BAKER, H. G. 1951. Hybridization and natural gene flow between higher plants. Biol. Rev., **26**: 302–337.

CARSON, H. L. 1951. Breeding sites of *Drosophila pseudoobscura* and *Drosophila persimilis* in the transition zone of the Sierra Nevada. Evol., **5**: 91–96.

CLARK, O. R. 1940. Interception of rainfall by prairie grasses, weeds and certain crop plants. Ecol. Monogr., **10**: 243–277.

DA CUNHA, A. B., H. BURLA, AND TH. DOBZHANSKY. 1950. Adaptive chromosomal polymorphism in *Drosophila willistoni*. Evol., **4**: 212–235.

DOBZHANSKY, TH. 1947a. A directional change in the genetic constitution of a natural population of *Drosophila pseudoobscura*. Heredity, **1**: 53–64.

——. 1947b. Adaptive changes induced by natural selection in wild populations of *Drosophila*. Evol., **1**: 1–16.

——. 1947c. A response of certain gene arrangements in the third chromosome of *Drosophila pseudoobscura* to natural selection. Genetics, **32**: 142–160.

——. 1948a. Altitudinal and seasonal changes produced by natural selection in certain populations of *Drosophila pseudoobscura* and *Drosophila persimilis*. Genetics, **33**: 158–176.

——. 1948b. Experiments on chromosomes of *Drosophila pseudoobscura* from different geographical regions. Genetics, **33**: 588–602.

——. 1949. Observations and experiments on natural selection in *Drosophila*. Proc. 8th Intern. Congr. Genetics, 210–224.

——. 1950. Origin of heterosis through natural selection in populations of *Drosophila pseudoobscura*. Genetics, **35**: 288–302.

——. 1951. Genetics and the Origin of Species. 3d Ed. Columbia Univ. Press. New York.

——. 1952. Changes induced by drought in *Drosophila pseudoobscura* and *Drosophila persimilis*. Evol., **6**: 234–243.

——, AND C. EPLING. 1944. Contributions to the genetics, taxonomy and ecology of *Drosophila pseudoobscura* and its relatives. Carnegie Inst. Publ. 554.

——, AND ——. 1948. The suppression of crossing over in inversion heterozygotes of *Drosophila pseudoobscura*. Proc. Natl. Acad. Sci., **34**: 137–141.

——, AND H. LEVENE. 1948. Proof of the operation of natural selection in wild populations of *Drosophila pseudoobscura*. Genetics, **33**: 537–547.

——, AND ——. 1951. Development of heterosis through natural selection in experimental populations of *Drosophila pseudoobscura*. Am. Nat., **85**: 247–264.

——, AND A. H. STURTEVANT. 1938. Inversions in the chromosomes of *Drosophila pseudoobscura*. Genetics, **23**: 28–64.

——, AND S. WRIGHT. 1947. Rate of diffusion of a mutant gene through a population of *Drosophila pseudoobscura*. Genetics, **32**: 303–324.

DOWDESWELL, W. H., R. A. FISHER, AND E. B. FORD. 1949. The quantitative study of Lepidoptera. 2. Maniola jurtina L. Heredity, **3**: 67–84.

DUBININ, H. P. 1948. Experimental investigation of the integration of hereditary systems in the process of evolution of populations. Zhurn. Obshch. Biol., **9**: 203–244.

EPLING, C., AND W. CATLIN. 1950. The relation of taxonomic method to an explanation of organic evolution. Heredity, **4**: 313–325.

——, AND H. LEWIS. 1952. Increase of the adaptive range of the genus *Delphinium*. Evol., **6**: 253–267.

GOLDSCHMIDT, R. B., A. HANNAH, AND L. K. PITERNICK. 1951. The podoptera effect in *Drosophila melanogaster*. Univ. Calif. Publ. Zool., **55**: 67–294.

HEUTS, M. J. 1948. Adaptive properties of carriers of certain gene arrangements in *D. pseudoobscura*. Heredity, **2**: 63–75.

HUMPHREY, R. R. 1933. A detailed study of desert rainfall. Ecology, **14**: 31–34.

LEVINE, R. P. 1951. Adaptive responses of some third chromosome types of *Drosophila pseudoobscura*. Evol., **6**: 216–233.

LEVITAN, M. 1951. Experiments on chromosomal variability in *Drosophila robusta*. Genetics, **36**: 285–305.

MATTSON, S., AND H. LÖNNEMARK. 1939. The pedography of hydrologic podzol series. I. Ann. Agric. College Sweden, **7**: 185–227.

MITCHELL, DONALD F., AND C. EPLING. 1951. The diurnal periodicity of *Drosophila pseudoobscura* in southern California. Ecology, **32**: 696–708.

MUSSON, C. T. 1903. Variations in rainfall at H. A. College. Agric. Gaz., New S. Wales. Publ. 644.

POTZGER, J. E. 1939. Microclimate and a notable case of its influence on a ridge in central Indiana. Ecology, **20**: 29–37.

ROWE, P. B., AND E. A. COLMAN. 1951. Disposition of rainfall in two mountain areas in California. Tech. Bull. U. S. D. A. 1048.

——, AND T. M. HENDRIX. 1951. Interception of rain and snow by second growth ponderosa pine. Trans. Am. Geophys. Union, **32**: 903–908.

SHREVE, F. 1931. Physical conditions in sun and shade. Ecology, **12**: 96–104.

WALLACE, B. 1952. The estimation of the adaptive value of experimental populations. Evol., **6**: 333–341.

——, AND J. C. KING. 1952. A genetic analysis of the adaptive values of populations. Proc. Nat. Acad. Sci., **38**: 706–715.

THE INTERACTION OF NUCLEUS AND CYTOPLASM IN THE ISOLATION AND EVOLUTION OF SPECIES OF PARAMECIUM [1]

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INTRODUCTION

The eight physiological species or varieties of the ciliated protozoan, *Paramecium aurelia,* are known to be genetically isolated from one another (Sonneborn and Dippell, 1946a and b; Sonneborn, 1947, 1950). This paper presents an analysis of the isolation of two of these varieties, varieties 4 and 8, chosen because of two rather unique features; their strong mating reactions with one another and their hybrid nonviability. The mixing together of cultures of the complementary mating types of the two varieties results, under optimum conditions, in mating reactions as strong as occur between the complementary mating types of either variety; yet the F1 generation is almost 100% nonviable. Between all other varieties of *P. aurelia,* mating reactions—if they occur—are much weaker and when they lead to conjugation generally result in high viability.

As will appear, the nonviability of hybrids between varieties 4 and 8 is due to a two way incompatibility between the cytoplasm of each variety and the nucleus of the other. In the extreme case, the nuclei may be destroyed prior to the death of the cell. Yet this destructive action of the cytoplasm is not invariable and a beginning has been made toward controlling it experimentally. Because of the occasional survival of nuclei in foreign cytoplasm, it has in effect been possible in a few cases to transform animals of one variety into the other variety. These results permit a preliminary view as to the evolution of these varieties and the nature of the mechanism that isolates them and preserves their integrity.

CYTOLOGICAL PROCESSES AND TERMINOLOGY

Knowledge of the cytological and other processes to be described below and the terminology to be used is essential for understanding what follows. The processes are common to all varieties of *P. aurelia.* They are conjugation, cytogamy, autogamy, macronuclear regeneration, cytoplasmic exchange, and selfing (Sonneborn, 1947).

With one exception each variety contains two complementary *mating types* designated by odd and even Roman numerals. The odd mating types of the different varieties are homologues as are the even ones. *Conjugation* occurs when cultures of complementary mating types are placed together under appropriate conditions. The initial reaction is one of clumping, following which pairs are formed. The same cellular processes take place in each member of a pair. The two micronuclei enlarge and each undergoes two meiotic divisions. Seven of the eight

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resulting haploid micronuclei disintegrate and the eighth remaining nucleus undergoes a single mitotic division. The two gamete nuclei thus formed are of identical genic constitution. One of these becomes the migratory micronucleus and passes through the *paroral* cone to fuse with the stationary micronucleus of its mate. The other gamete nucleus remains stationary and receives the migratory micronucleus of its mate. Following the formation of the syncarya, the two mates separate. Since the gamete nuclei fusing in one mate are sister nuclei of those in the other mate, it is evident that the two mates must have syncarya with identical genic constitutions.

Cytogamy is essentially similar to conjugation except that no nuclear exchange takes place between the mates. Both members of such a pair undergo self-fertilization, the syncaryon being formed by the fusion of sister haploid micronuclei of identical genic constitution. The syncaryon must be homozygous for all its genes.

Autogamy occurs periodically in all stocks of *P. aurelia*. This process is identical with cytogamy except that it occurs in single unpaired animals.

In all three processes, the syncaryon divides twice mitotically, producing four products, two of which become micronuclei and two of which develop into macronuclei. The anlagen of the two macronuclei are regularly segregated into different cells at the first postzygotic cell division and develop into mature macronuclei. Cells of the same genotype, whose micronuclei are derived from a single syncaryon, are called a *clone*.

The fate of the old macronucleus is the same in all three processes. The macronucleus breaks down through a skein stage into 30 to 40 fragments, which are passively distributed at random to the subsequent fission products and are eventually resorbed.

Sonneborn (1940) has reported that under certain conditions the macronuclear anlagen fail to develop or fail to segregate normally, producing cells in which no anlagen are present. In such cells the fragments of the old macronucleus may fail to disintegrate, in which case they grow while they are being segregated into the daughter cells until only one is present. These fragments are now the size of the normal macronucleus and divide at each cell division thereafter. This process of the development of new macronuclei from the fragments of the old macronucleus is known as *macronuclear regeneration*. Sonneborn concluded from these and other facts that each fragment of the macronucleus contains at least one set of genes.

Conjugating paramecia sometimes exchange cytoplasm. Cytoplasmic bridges, persisting for varying lengths of time, are evident in the region of the paroral cones after the exchange of nuclei. These bridges are indicative of the exchange of cytoplasm between the conjugants. The quantities of cytoplasm exchanged vary roughly with the duration and size of the bridges (Sonneborn, 1944).

Conjugation occasionally occurs between the cells of a clone. Such clones, called *selfers*, include animals of both mating types.

Materials. The Stocks of Varieties 4 and 8: Distinctions and Distribution

All the stocks in Sonneborn's collection belonging to varieties 4 and 8 have now been classified and none of these fails to conform to the criteria (to be presented in the methods section) for one or the other of the two varieties. The accompanying tables (1a and 1b) list the stocks according to variety, the place collected, the collector, and the year collected. Except for the three groups of stocks enclosed in brackets (table 1b), each stock was started with a single animal isolated from each collection. The stocks in a given group in brackets are difference isolates from the same collection bottle, but are genetically diverse. (It is not known whether stocks 131–2 and 131–4 are genetically different from each other; how-

TABLE 1a. *The stocks of variety 4*

Stock	Place collected	Collector	Year
29	Hebbville, Maryland	Kimball	1938
32	Towson, Maryland	Kimball	1938
47	Berkeley, California	Giese	1939
51 K[1]	Spencer, Indiana	Clemmer	1939
111	Between Halethorpe and Montgomery Road on Baltimore–Washington Boulevard, Maryland	Preer	1946
116 K	Bloomington, Indiana	Butzel	1949
126	Richomnd, Florida	Gilman	1947–8
127	Richmond, Florida	Gilman	1947–8
128	Richmond, Florida	Gilman	1947–8
139 K	Ft. Lauderdale, Florida	L. and D. Sonneborn	1946
146	Honshu, Japan	Hiwatashi**	
163	Valley Forge, Pennsylvania	Preer	1947
169 K	Morioka City, Japan	Hiwatashi**	
170	Yamagata, Japan	Hiwatashi**	
171	Suzukawa, Japan	Hiwatashi**	
172	Macchu Picchu, Peru	T. M. Sonneborn	1951
173	El Tabo, Chile	T. M. Sonneborn	1951
174	Valparaiso, Chile	T. M. Sonneborn	1951

TABLE 1b. *The stocks of variety 8*

Stock	Place collected	Collector	Year
31	Montgomery Road and Washington Boulevard, Maryland	Kimball	1938
(130 MK[2]	Ft. Lauderdale, Florida	Gilman	1947
(130–3	Ft. Lauderdale, Florida	Gilman	1947
[131 MK	Ft. Lauderdale, Florida	Gilman	1947
[131–2*	Ft. Lauderdale, Florida	Gilman	1947
[131–4*	Ft. Lauderdale, Florida	Gilman	1947
(137	Ft. Lauderdale, Florida	L. and D. Sonneborn	1946
(138 MK	Ft. Lauderdale, Florida	L. and D. Sonneborn	1946
(141	Ft. Lauderdale, Florida	L. and D. Sonneborn	1946
150	Cypress Gardens, Florida	Gilman	1948
151	Bok Tower, Florida	Gilman	1948

[1] K = Killer.

[2] MK = Mate killer.

* These stocks have not yet been tested against variety 3.

** These stocks were sent to Sonneborn by Hiwatashi. It is not known for certain that he collected them.

ever, they are both different from stock 131.)

Variety 8 is not nearly as cosmopolitan as variety 4. Except for one collection in Maryland, all the other stocks of variety 8 were found in Florida. On the other hand, variety 4 is found not only throughout the United States, but appears widespread in South America and Japan. Stocks of variety 4 have been found in both the localities known to be inhabited by variety 8. Stock 111, variety 4, was collected in Maryland near where stock 131, variety 8 was found. Stocks 137, 138 and 141, variety 8 were all isolates from the same collection bottle as was stock 139, variety 4.

Four of the stocks of variety 4 (stocks 51, 116, 139, and 169; table 1a) show the killer trait (Sonneborn, 1943). Killers liberate into the fluid in which they live a poison, paramecin, which kills sensitive

animals. Sonneborn and his colleagues (Sonneborn, 1943, 1947; Preer, 1948, 1950; Dippell, 1950) have shown that the production of paramecin depends on the presence in the cytoplasm of desoxyribose nucleoprotein particles called kappa; which in turn persist only in the presence of a dominant gene K. Three stocks of variety 8 (stocks 130, 131, and 138; table 1b) exhibit the mate-killer trait (Siegel, 1953 a and b; Levine, 1953), which is identical in its genetics with the killer trait of variety 4. Mate-killing depends on a gene, M, and cytoplasmic particles called mu. As a result of the prolonged contact necessary for the completion of conjugation, paramecia sensitive to mate-killing die when crossed to these three stocks. The killer trait is restricted in its distribution to varieties 2 and 4 and the mate-killer trait to variety 8.

Killers and mate-killers have been found together in nature and in close association with sensitives. Stock 139 killers and stock 138 mate-killers were descended from isolates from the same collection bottle as were stocks 137 and 141 sensitives. Stock 130 mate-killers were derived from an isolate from the same collection bottle as were stock 130–3 sensitives. Stock 131 mate-killers were obtained from the same bottle as were stocks 131–2 and 131–4 sensitives. The proximity of killers and mate-killers and sensitives in nature may be indicative of the ineffectiveness of these traits as agents of selection against sensitives in their natural surroundings.

Sensitives, environmentally derived from the three mate-killers by the removal of mu from the cytoplasm, but isogenic with them (Siegel, 1953b and Levine, 1953), were also used in parts of the analysis to be presented.

Methods

The methods employed in the handling and crossing of the materials are those described by Sonneborn (1950). Only the general techniques for classifying the stocks of varieties 4 and 8 and for the determination of viability will be presented here. Other techniques will be described when necessary. Except when otherwise mentioned, all cultures were grown at 27° C.

Method of classifying the stocks of varieties 4 and 8

An unknown culture of variety 4 or 8 can be classified on the basis of its breeding relations with known stocks of varieties 3, 4, and 8 (Sonneborn and Dippell, 1946b; Sonneborn, 1950). If an unclassified culture shows strong mating reactions with the stocks of both varieties 4 and 8, if the F1 from the cross with variety 8 shows practically 100% viability while the F1 from the cross to variety 4 shows about 95% nonviability, and if its even mating type mates with mating type V of variety 3, the culture is classified as belonging to variety 8. If, however, it shows 100% F1 viability with variety 4 and nearly 100% nonviability with variety 8, and shows no reactivity with variety 3, it is classified as belonging to variety 4. These breeding relations are summarized in table 2.

The method of determination of viability

The primary character followed in this analysis was the viability or nonviability of exconjugant or exautogamous clones. A clone was classified as viable, *V*, if it exhausted all the food available in about 0.75 ml. of culture fluid in three to six days and completed at least 10 fissions in the process. The classification as a viable or surviving clone indicates nothing as to the type of nuclear reorganization (normal reorganization or macronuclear regeneration) which gave rise to the clone, the viability of all the sublines of the clone during vegetative reproduction, or the viability of the progeny of the clone following the next nuclear reorganization. A nonviable clone, *N*, was one which died before it completed 10 fissions following nuclear

TABLE 2. *The system of breeding relations of varieties 3, 4, and 8*

The numbers in the table above the diagonal give the maximum percentage of animals that agglutinate in mixtures of the mating types in the corresponding row and file. The abbreviation "Inc" stands for the incomplete mating reactions shown by most of the animals in these mixtures, which do not lead to conjugation. The numbers below the diagonal give the average percentage of viable offspring from conjugant pairs between the two mating types. The question mark indicates that the frequency of viable offspring following the cross of mating type V to XVI had not been determined. (Adapted from Sonneborn, 1947 and 1950; and personal observations.)

Variety		3		4		8		
	Mating type	V	VI	VII	VIII	XV	XVI	
3	V	0	95	0	0	0	40	Inc
	VI	100	0	0	0	0	0	
4	VII			0	95	0	95	Inc
	VIII			100	0	95	0	
8	XV				5	0	95	
	XVI	?		5		100	0	

reorganization. Such clones may pass through several fissions before they die or may not undergo any fissions.

THE CROSS BETWEEN VARIETIES 4 AND 8

The remarkable features of the cross between the stocks of varieties 4 and 8 were previously noted by other investigators (Sonneborn and Dippell, 1946a and b; Sonneborn, 1950) using stock 31, variety 8, and stocks 29, 32, 47, and 51 of variety 4. Melvin (Master's thesis, 1949, unpublished) studied the cross of stock 31, variety 8, to stock 51, variety 4. In the course of the work reported in the present paper, the intervarietal breeding relations of all the known stocks of the two varieties were examined, many of which were not available to earlier investigators. The characteristic peculiarities of the intervarietal cross appear first in the mating reaction and occur in many of the stages thereafter.

The mating reaction

The initial mating reaction between the stocks of the two varieties are as strong as those occurring within the varieties (Sonneborn and Dippell, 1946a and b). When animals of mating type VIII, variety 4, are mixed with those of mating type XV, variety 8, most of the animals that clump eventually form pairs. In the reciprocal cross, using mating types VII and XVI, far fewer animals form pairs than clump. An agglutination reaction in which 95% of the animals clump, may produce less than 10% pairs (table 2). However, with enough material, large numbers of pairs can be obtained with ease from both mixtures.

The results of conjugation

The duration of the process of conjugation is the same as in the intervarietal crosses. Conjugation is completed in five and one-half to six hours at 27° C. and appears normal. A cursory cytological study of the cross of stock 51 to stock 131 indicated that all nuclear processes were generally normal except for the occurrence of some cases of macronuclear regeneration in some of the survivors of the intervarietal cross.

Isolated and grown in separate slide depressions, the exconjugants were individually observed. Two days after conjugation, the animals appeared normal, but one exconjugant divided at a slower rate than the other. The more rapidly dividing exconjugant usually passed through five fissions, as had the intravarietal controls, while the other slower exconjugant completed only two to three fissions. The next day both exconjugants usually completed four to six fissions and as a rule divided no more. The animals became dark, very thin, and after a week eventually died. Melvin (1949) never found a viable F1 clone from the intervarietal cross. Sonneborn (unpublished) and this

writer have occasionally found viable F1 clones. These will be discussed in greater detail.

The hybridity of the nonviable exconjugants

Melvin's (1949) primary contribution was to provide the best evidence available for reciprocal fertilization and the hybridity of the F1. Melvin crossed cultures marked with different serotypes (Sonneborn and LeSuer, 1948). The stock 51 animals were marked with serotype A and the stock 31 animals were marked with a variety 8 serotype against which she obtained serum. These cultures were mated and the exconjugants tested for their serotypes after the first fission. One exconjugant of each pair tested as stock 51 serotype A and the other as the stock 31 serotype. After the exconjugant clones had undergone five to six fissions they were again tested for serotypes and now both exconjugant clones of a pair were of the stock 31 serotype. The exconjugant clones derived from stock 51 had transformed to the serotype of stock 31. Melvin attributed the change of serotype to the introduction of the stock 31 nuclei into the stock 51 animals since the change did not occur if the conjugants were forced apart before fertilization could take place. Sonneborn (unpublished) has observed similar results in intervarietal crosses between varieties 4 and 8. As a consequence of marking the animals with serotypes, Melvin showed that the exconjugant which divided at the slower fission rate was cytoplasmically descended from stock 51.

The cause of exconjugant death in the intervarietal cross

Melvin (1949) concluded that, although there was evidence of nuclear exchange in the cross between stocks 31 and 51, the effects of "cytoplasmic contact alone" are enough to set up a reaction that affects the nuclei and results in the death of the conjugants. This conclusion was based on two lines of evidence.

First, Melvin exposed intervarietal pairs to mixtures of homologous antisera to induce cytoplasmic exchange (Sonneborn, 1950). Death of both conjugants of these pairs, supposedly due to the mixture of the cytoplasms, occurred before they could separate. These findings were held to demonstrate the involvement of the cytoplasm in the intervarietal mortality, since death ensued sooner than when cytoplasms were not exchanged.

Second, Melvin forced apart the conjugants of four intervarietal pairs after five hours of conjugation, but before the nuclei could exchange. These animals survived longer than the animals allowed to complete conjugation, but still died. The death of these animals without nuclear exchange and the report of a lethal intervarietal contact effect in *Paramecium bursaria* led Melvin to the conclusion that contact alone was enough to cause the mortality in the variety 4 by 8 cross. Chen (1946b) showed that in certain intervarietal crosses of *P. bursaria,* the micronuclei did not develop beyond the first prezygotic division and the animals died without nuclear exchange. He interpreted this as due to diffusion of substances across the membrane.

Three findings minimize mere contact of the diverse cytoplasms as a factor in the intervarietal mortality. (1) As will be reported below, an occasional cytogamous pair survived the cross of variety 4 to 8. Although the mates were in intimate contact for the time it takes to complete conjugation, one member of these pairs gave rise to normal variety 4 animals and the other to normal variety 8 animals. (2) Experiments will be reported in which crosses were made between amicronucleate animals of one variety and micronucleate animals of the other variety. The clones descended from the micronucleates generally survived the contact with the amicronucleates. (3) Similarly, a third animal which makes and maintains contact, throughout conjuga-

tion, with the animal of complementary mating type (and the other variety) in intervarietal pairs, will also survive. Chen (1946a) reported unions of three animals in *P. bursaria* and showed that two conjugate while the third, which may attach in any position to the conjugant of complementary mating type, undergoes autogamy. In our work, of the 21 clones derived from seven unions of three animals in mixtures between stock 51 of variety 4 and stock 131 of variety 8, twenty survived and produced normal cultures of their respective varieties. The one fatality was clearly due to injury sustained during manipulation. In this cross, unlike Chen's, the union of three animals interfered with conjugation so that all three animals underwent cytogamy. The results thus show again that there is no diffusion of a lethal substance across cell membranes and that mere prolonged contact cannot be responsible for intervarietal nonviability.

Nevertheless, Melvin was correct in concluding that the cytoplasm plays an important role in the intervarietal lethality. This role will be demonstrated and discussed below, but not before the analysis of the F1 survivors of the intervarietal crosses is presented.

The Analysis of the Viable F1 Clones from the Cross of Variety 4 to 8

The exciting prospect about the survivors of the intervarietal cross is their possible hybridity. It was hoped that a breeding analysis of the survivors would establish their hybridity and provide some clues as to the cause of mortality in the F1. Not all the survivors, however, need to be true hybrids. It will be necessary first to characterize the survivor clones and then decide whether these characteristics permit classification of the survivors as true or false hybrids.

Since the frequency of survivors was the same in all the intervarietal combinations of stocks examined, the analysis was restricted to those arising from the reciprocal crosses of stock 51 killers, variety 4, to stock 131 sensitives, variety 8. Stock 51 was used so that the killer trait could serve both as a cytoplasmic marker for detecting the survivors descended from variety 4 and as a trait to be followed for genic segregation in later generations. Stock 131 was chosen because it gave very little mortality at autogamy.

The viability of the F1 generation of a number of crosses of stock 51 to stock 131 is listed in table 3. Of the 726 exconjugant clones included in table 3, only 48 survived. The survival rate was 6.6%. However, only the crosses that produced survivors which were analyzed are included in this table; many others did not produce survivors and the survivors of

TABLE 3. *The viability of the F1 exconjugant clones of the intervarietal reciprocal crosses of stock 51 killers to stock 131 sensitives*

Cross I. 51 VII killers × 131 XVI sensitives

	No. prs.	V–V*	V–N**	N–N***
A	60	6	2	52
C	30		1	29
D	15		1	14
E	15	5	1	9
G	15			15
H	30	1	2	27
Totals	165	12	7	146

Cross II. 51 VIII killers × 131 XV sensitives

	No. prs.	V–V	V–N	N–N
A	60		1	59
B	15	1	1	13
C	30	1		29
D	15			15
E	3		1	2
F	30	1	1	28
G	15		1	14
H	30	2	2	26
Totals	198	5	7	186

* V–V = Both exconjugants of a pair viable.
** V–N = One exconjugant of a pair viable; the other nonviable.
*** N–N = Both exconjugants of a pair nonviable.

others were not analyzed. When all the crosses are taken into account, the survival rate is about 5% (table 2).

Methods for characterizing the viable F1 clones

Four tests were employed to characterize the viable F1 clones, the purpose of the first two being the determination of the cytoplasmic origin of the survivors. As one of the parents was a killer and the other a sensitive, and as killing is cytoplasmically inherited (Sonneborn, 1943), the survivors showing killing activity were assumed to be descended from variety 4 and the sensitives from variety 8. Since the parent conjugants always differed in mating type and since mating type seldom changes following conjugation (Sonneborn, 1942, and Nanney, 1953, for variety 4; Siegel, 1953b, and Levine, 1953, for variety 8), the mating type of the survivors was determined as a corroborating test for their cytoplasmic origins. There was a very high correlation between the mating types of the variety 4 parents and that of the killer survivors on the one hand and the mating type of the variety 8 parents and that of the sensitive survivors on the other hand. No trouble was encountered in determining the varietal origins of any of these survivors.

Two other tests were designed to distinguish true from false hybrids. One test employed the 95% mortality in the F1 generation of the intervarietal cross as compared with practically no mortality in the intravarietal crosses (table 2). Accordingly, the survivors were backcrossed to stocks of both varieties. If the progeny of one backcross gave practically 100% viability, the survivor was indistinguishable by this test from the stock to which it was crossed. This could be confirmed by 95% mortality in the cross to the stock of the other variety. If both backcrosses resulted in high mortality, this could indicate a hybrid constitution. A corroborating test for hybridity was to detect mortality at the first autogamy in survivor clones. A true hybrid might be expected to give very high mortality among the autogamous F2, as this happens even in the autogamous F2 of crosses between stocks of the same variety in both variety 4 and 8.

Thus, a survivor clone which shows no mortality at autogamy and in the backcross to the parent from which it is cytoplasmically descended, but high mortality with the other parent, obviously could not be a hybrid. These results are expected of a clone pure for the genes of one of the parental stocks. A true hybrid would show mortality following genic recombination. Should the stocks of the two varieties differ in chromosome number and/or structure, normal pairing and disjunction would not be expected to take place, also giving rise to mortality.

But mortality in the backcrosses of the survivors or at autogamy could be due to causes other than a hybrid genome. Many nuclear aberrations would give results similar to those expected of a hybrid. Haploids and aneuploids, otherwise pure for the genes of a single stock, would result in mortality at autogamy and in crosses. Such nuclear abnormalities may be superimposed on the hybrid genome and further complicate the situation. Until the chromosomes of both varieties are genically marked or cytologically distinguished, segregation of a genetic trait in intervarietal crosses is the only available index of hybridity of the survivors. Even this index may be complicated by the loss of chromosomes. Thus it is apparent that the outlined tests can merely detect certain nonhybrid types and leave the nature of the other survivors in a state of uncertainty.

The two classes of viable F1 clones

The application of these tests to the 48 survivors listed in table 3 resulted in two classes of survivors. However, before going into the analysis of the survivors, a few preliminary comments are in order. Whenever an intervarietal cross was

made, intrastock exconjugants were followed as controls and were observed for viability at the first autogamy. Intervarietal control crosses were made and the exconjugants followed whenever backcrosses were made. These control crosses are not described below because they produced the expected results and these have already been amply described.

In crosses F through H (table 3), the exconjugant clones were examined for the occurrence of macronuclear regeneration. The exconjugant clones were expanded through the second fission into *quadrants*. Third fission products were stained and examined for the presence or absence of newly developed macronuclei. Macronuclear regeneration was found only in some of the clones that acted in the backcrosses and at autogamy as would be expected of hybrids. The occurrence of macronuclear regeneration in these survivors was the only deviation from normal cytological processes known in the intervarietal crosses. As a result of expanding the exconjugant clones through the third fission in order to make this examination, it was found that not all the sublines of a clone were equally viable.

Class 1. The viable clones of class 1 were not hybrids. These clones behaved as normal animals of one or the other variety in backcrosses and at autogamy. Of the 17 cases of the survival of both members of a pair (table 3), eight pairs were cases of selfing between stock 51 animals and five pairs were cases of cytogamy between animals of stock 51 and 131. These account for 26 of the 48 survivors listed in table 3. The total may be raised to 29 since in three other pairs, one exconjugant behaved in the backcrosses and at autogamy as would be expected of nonhybrids, thus falling into this class; while their mates behaved like the clones of class 2.

Class 2. The 19 remaining surviving clones showed mortality at autogamy and in the backcrosses to *both* parents. Three of these were those mentioned in the previous paragraph whose mates were nonhybrid. Two other survivors were members of the same pair, both of which behaved as would be expected of hybrids. Fourteen were cases of single surviving exconjugants. The death of the mates of these 14 clones indicates that a hybrid genome may have been formed in at least one member of these pairs and makes these survivors the most promising from the point of view of possible hybridity.

The F1 survivors of this class are not a homogeneous group. (1) The survivors were phenotypically heterogeneous in that the phenotypes of all four parental types were represented. (2) There were differences in the viability of the progeny of these clones in the backcrosses and at autogamy. (3) Some survivors arose by normal reorganization and some by macronuclear regeneration. (4) The number of micronuclei per cell differed in the clones descended from different survivors and even to some extent among the cells of a given clone. Most of the cells of these clones had two micronuclei, but some appeared to be amicronucleate. Most of the cells of one of these clones were, however, unimicronucleate. The rest were amicronucleate.

Since abnormal conjugants occur sometimes even in intravarietal crosses, the question arises as to whether the survivors of class 2 are simply derived from ordinary nonhybrid abnormal conjugants. There is reason to believe that this is not the case. (1) The frequency of abnormal clones arising from the intravarietal crosses was much lower than that of the clones of class 2. (2) Large numbers of intravarietal control exconjugants were examined for macronuclear regeneration, since it was found that a large proportion of the clones of class 2 reorganized in that manner. No definite case was found in either variety and the few doubtful cases proved to be viable at the first autogamy and in intravarietal crosses. (3) As a consequence of the examination for macronuclear regeneration it was found that a small number of the intravarietal quadrants were nonviable. Their viable sister

quadrants were tested and found to be normal. Thus it seems highly unlikely that these survivors of the intervarietal crosses were abnormal lines of the kinds which regularly occur in the parental stocks. They were nonviable in the backcrosses and at autogamy because of something which happened as a result of the intervarietal cross.

After the first autogamy in clones of class 2, those cells which did not undergo macronuclear regeneration died within the first few fissions; whereas those which did undergo macronuclear regeneration, lived for varying lengths of time, but eventually died. Cytological preparations of some of the viable autogamous lines showed that most of the cells of these cultures were amicronucleates, with a few unimicronucleates and bimicronucleates. Thus, the cells of viable autogamous clones usually manifest abnormal micronuclear conditions and depend for survival upon products of the same macronucleus which proved capable of supporting life in the F1. The widespread occurrence of macronuclear regeneration precludes detecting Mendelian segregation at autogamy, and the lack of micronuclei makes these animals of no further genetic use.

The prevalence of macronuclear regeneration among the F1 and autogamous F2 survivors suggested that macronuclear regeneration might be common among the viable progeny of the backcrosses. Therefore, it was surprising to discover that the frequency of macronuclear regeneration in the backcrosses was as low as in the original intervarietal cross. Since most of the backcross survivors cross by normal nuclear reorganizations, genic segregation (if it occurs) should be found by backcrossing. However, genic analysis cannot be extended to autogamy among the backcross progeny, for the mortality rate among these was no lower than among the viable autogamous clones from F1 survivors. In both cases survival depended upon macronuclear regeneration.

Twice F1 killer survivors were tested for their hybridity by attempting to show segregation of the killer trait in backcrosses. First, a mating type VII killer clone was backcrossed to sensitives of stock 131; but no segregation of the killer trait occurred among the 12 clones which were cytoplasmically descended from it. All 12 remained killers. Second, a mating was made between two surviving F1 killer clones of opposite mating types. Again segregation failed to occur. Of 60 pairs followed, only 20 exconjugant clones survived and all remained killers.

Three possible explanations for the lack of segregation may be offered. First, the F1 killer survivors may not have been hybrids. Second, the F1 survivors may have been hybrid, but stock 131 may have genes which can support the killer trait. Third, since the nuclear condition of at least some survivors is known to be abnormal, these survivors may have been incapable of undergoing the normal nuclear processes necessary for the detection of Mendelian segregations. None of these explanations can be excluded by available data.

The Experimental Increase of F1 Survival in the Variety 4 by Variety 8 Cross

The survival of some clones in class 2 following macronuclear regeneration suggested that the frequency of F1 survival in the intervarietal cross might be increased by experimentally increasing the frequency of macronuclear regeneration. The nonhybrid macronucleus of the parent might protect the newly formed hybrid micronucleus of the F1. The surviving animals might then be tested, in the manner previously described, for mortality at the first autogamy and in backcrosses and the results compared to those expected of hybrids and those obtained from the naturally occurring survivor clones of class 2.

Three crosses were made: stock 51 VIII killers × 131 XV sensitives, stock 51 VII killers × VIII killers, and stock 131 XV sensitives × XVI sensitives. Pairs from these three crosses were allowed to

complete conjugation at 27° C. Each of the three sets of pairs was then split into two groups: a control group maintained at 27° C. following conjugation and for the duration of the experiment; and an experimental group placed in three mass cultures in preheated culture fluid in a water bath at 36° C. immediately after completion of conjugation to induce macronuclear regeneration (Sonneborn, 1950). The latter pairs were kept at 36° C. for two hours; such treatment prevents the normal development of macronuclear anlagen in a large number of the exconjugants.

The viability of the exconjugant clones of the control groups, not subjected to high temperature, was as expected. Fifteen pairs were followed in each intravarietal cross and all the progeny were viable. All the exconjugant clones of the 29 intervarietal pairs followed died after completing four to six fissions. These clones were not examined for macronuclear regeneration as it does not occur to any considerable extent at 27° C.

The data on the survival of the heat-treated clones are given in table 4. It was not possible to follow the individual pairs as these animals were treated in mass cultures. After the two-hour heat treatment, single exconjugants were isolated and followed.

TABLE 4. *The viability of the F1 exconjugants of the intervarietal and intravarietal crosses exposed to 36°C. for two hours immediately following the completion of conjugation*

Crosses	Viability of exconjugant clones				
	No. clones	V*	N**	%V	%N
A. 51 VIII killers × 131 XV sensitives	60	33	27	55	45
B. 51 VII killers × VIII killers	30	23	7	77	23
C. 131 XV sensitives × XVI sensitives	30	21	9	70	30

* V = viable.
** N = nonviable.

More than half of the heat-treated clones from the cross of stock 51 to stock 131 survived as compared to no survival among the non-heat-treated intervarietal clones. The increased survival was over and above the lethal effect of the heat treatment as measured by the death of some of the heat-treated intravarietal exconjugant clones. Thus, in spite of the fact that heat itself resulted in some nonviability, heat treatment increased the frequency of survival of the intervarietal exconjugants. How can this be explained? At least one and possibly two factors seem to be involved.

First, macronuclear regeneration occurred in most of the survivors. Fifteen exconjugants from each one of the three crosses listed in table 4 were expanded and stained for macronuclear regeneration. Of the 25 surviving quadrants from the intervarietal clones, 17 were macronuclear regenerates, four had passed through normal reorganization, and four were either undetermined or questionable. Of the 30 surviving quadrants of the stock 51 VII × VIII cross, 15 were macronuclear regenerates, 10 were normal and five were questionable. In the intrastock 131 cross, 21 were macronuclear regenerates, 15 were normal and one was questionable of a total of 37 surviving quadrants.

The majority of the surviving quadrants listed above were backcrossed to at least one of the parental stocks, to test for viability, in all cases at least to the stock from which they were immediately derived. Twenty-three of the above mentioned 25 intervarietal heat-treated quadrants were backcrossed and in all cases showed high nonviability. That is they acted like the survivors of class 2, in these backcrosses. Twenty-eight quadrants of the heat-treated intravarietal stock 51 were backcrossed to stock 51 and only eight showed any nonviability. Twenty-nine of the stock 131 quadrants were backcrossed to stock 131 and only six of the backcrosses showed any nonviability. The nonviability in the backcrosses of the intravarietal clones was much less than that

found in the backcrosses of the intervarietal clones, and was probably due to abnormalities resulting from the heat treatment. The higher mortality found in all of the backcrosses of the intervarietal clones must be mainly due to the fact that they came from an intervarietal cross and may be a result of hybridity.

The second factor in the increased survival may be a more direct effect of heat treatment than that of inducing macronuclear regeneration. Heat may be inactivating some substance or substances involved in the lethality resulting from the cross of variety 4 to 8. This is indicated by the fact that the frequency of normally reorganizing surviving exconjugants which manifested high nonviability in backcrosses was much greater following heat treatment than ordinarily. In the heat-treated series, there were two in a group of 15; in all the work at 27° C., there were two in a group of 240. Evidence for substances causing the mortality in the intervarietal crosses will be presented in the next section. Further evidence of the lability of the lethal interaction will be presented in another connection. A careful analysis of the action of many environmental and chemical agents on the intervarietal lethality should be made.

Although there is no absolute proof of the hybridity of any of the survivors of the intervarietal crosses, there is reason to believe that the heat-treated survivors are hybrids. These would have died in typical intervarietal fashion but for the heat treatment. The similarity of the results of the backcrosses of both the heat-treated survivors (these were not examined for viability at autogamy) and the naturally occurring survivors of class 2 might be taken as suggestive of the hybridity of the latter. The hybrid nature of both types of survivors might be established by repeating the crosses, using variety 4 stocks now available in Dr. Sonneborn's laboratory, containing multiple gene markers. These survivors could then be backcrossed with the greater expectation of finding segregation of at least some of the traits. Care must be exercised in the interpretation of such results in view of the known abnormal nuclear condition of some of the naturally occurring survivors.

Evidence for a Nucleo-Cytoplasmic Incompatibility System Causing the F1 Mortality in the Cross of Variety 4 to 8

The basis of the mortality following crosses between the stocks of varieties 4 and 8 was analyzed by crosses closely akin to the merogony experiments of the embryologists. Amicronucleates of each variety were mated to micronucleates of the other. Such crosses have certain advantages over the intervarietal micronucleate matings. (1) The amicronucleates can only accept genes, but cannot contribute any; thus these crosses cannot be complicated by the formation of hybrid genomes. It should then be theoretically possible to determine whether the genes of one variety can function normally in the cytoplasm of the other variety. This is a typical merogony experiment. (2) Since conjugating paramecia sometimes exchange cytoplasm it is possible in these crosses to observe the effect of introducing into one variety varying amounts of cytoplasm from the other variety, without the complication of introducing genes also. The latter situation, brought about here easily and naturally, could be accomplished only by micro-injections in higher organisms.

For this work, amicronucleate cultures of mating types VII and XV were employed. The type VII culture, a killer of stock 51, was provided by Dr. Henry M. Butzel, Jr. who obtained it by treating conjugating stock 51 animals with barely sublethal doses of colchicine (Butzel, 1953). The type XV (variety 8) amicronucleates, a culture of stock 138 sensitives, were provided by Dr. R. W. Siegel who obtained them by diluting out the mu particles of a mate-killer at 32° C. As set forth by Siegel (1953b), when mate-

TABLE 5. *Summary of viability of the exconjugants of the intravarietal and intervarietal matings of amicronucleates to micronucleates*

Crosses	No. prs.	Clones derived from amicronucleate mate		Clones derived from micronucleate mate		Totals	
		V**	N***	V	N	V	N
A. 51 VII killer amicronucleates ×variety 8 XVI sensitives*	316	2(0.6%)	314(99.4%)	150(47%)	166(53%)	152(24%)	480(76%)
B. 138 XV sensitive amicronucleates ×51 VIII killers	248	1(0.8%)	247(99.2%)	229(92%)	19(8%)	230(46%)	266(54%)
C. 51 VII killer amicronucleates ×51 VIII killers	214	60(28%)	154(72%)	198(93%)	16(7%)	258(60%)	138(40%)
D. 138 XV sensitive amicronucleates ×138 XVI sensitives	224	101(45%)	123(55%)	221(99%)	3(1%)	332(72%)	126(28%)

* Three variety 8 sensitives (stocks 130, 131, and 138) were used in this cross.
** V = viable.
*** N = nonviable.

killers of stock 138 lose their mu particles, their micronuclei are damaged and often lost.

Different amicronucleate cultures, and even the same culture at different ages, are not necessarily identical in viability. Although the two amicronucleates used in this work were not exactly alike, they were both highly viable and the work to be reported was completed before any aging effect was noted. Further, they were of homologous mating types.

The data to be presented were obtained over a period of three months and are presented in pooled form (table 5). Before discussing the results of the intervarietal crosses, an examination of the intravarietal control crosses of amicronucleates to micronucleates will be made.

The intravarietal crosses of amicronucleates to micronucleates

In these crosses, the amicronucleate conjugants frequently yielded viable progeny (C and D, table 5). That these survivors received functional micronuclei was shown by the presence of macronuclear anlagen in the exconjugants and by the presence of micronuclei in permanently stained samples of these clones. The vast majority of the micronucleate conjugants also yielded viable progeny.

Survivors of such crosses should initially be haploids, as each exconjugant forms its new nuclear apparatus from one of the two haploid gamete nuclei of the micronucleate conjugant. The test used for evidence of haploidy was the occurrence of mortality at the next nuclear reorganization. This test was based on the expectation that a haploid could not readily produce a balanced genome at meiosis. Most of the viable F1 showed the expected mortality at the next fertilization. The survival of almost all the F1 indicates that haploidy itself has little if any direct detrimental effect. These F1 clones, even those from the parent of odd mating type were usually of the even mating types; that is, the clones descended from the amicronucleates changed mating type in agreement with the findings of others (Sonneborn, unpublished; Levine and Siegel, unpublished).

The intervarietal crosses of amicronucleates to micronucleates

Three different variety 8 stocks, 130, 131 and 138, were used in the matings to the amicronucleates of stock 51. All gave similar results. A considerably greater proportion of the progeny survived the intervarietal crosses of amicronucleates to micronucleates (crosses A and B, table 5), than survived the crosses between micronucleate cultures of the two varieties. The increased survival was primarily due to the viability of the offspring derived from the micronucleate member of each pair. Among the survivors in each intervarietal cross, only two F1 clones in each cross were probably descended from the amicronucleate members. (Three are listed in table 5, the fourth in table 7.) These will be discussed later.

The viability of the progeny of the micronucleate conjugants of the cross should not be surprising. Since the micronucleates in these crosses did not receive any nuclei from the amicronucleates, and since there is no evidence of a lethal contact effect, one might expect no more mortality here than occurred in the progeny of the corresponding member of the intravarietal crosses (crosses C and D, table 5). However, only 47% of the progeny of the micronucleate conjugants of the cross of stock 51 killer amicronucleates to variety 8 sensitives (cross A, table 5) were viable as compared to 92% survival of the progeny of the micronucleate conjugants of the other intervarietal cross (cross B, table 5).

The explanation of the difference in viability of the progeny of the micronucleates came from an examination of the process of conjugation in the two intervarietal crosses. Many of the conjugating pairs from the cross of stock 51 killer amicronucleates to variety 8 sensitives showed very narrow cytoplasmic bridges in the paroral cone region and these bridges were of short duration, lasting *two* to *three* minutes, indicative of the exchange of small amounts of cytoplasm. The micronucleate conjugants of 83% of the pairs showing these cytoplasmic bridges died (table 6, cross A). Very few of the pairs from the cross of stock 138 amicronucleates to stock 51 killers showed these cytoplasmic bridges, in agreement with the viability of the progeny of the micronucleate conjugants (table 6, cross B).

Thus it appears that the introduction of cytoplasm from the stock 51 amicronucleate causes the death of its variety 8 micronucleate mate. If this assumption is correct, then the induction of cytoplasmic exchange in cross B (table 6) where it did not regularly occur, should result in

TABLE 6. *The correlation of cytoplasmic exchange with the viability of the exconjugants of the intervarietal crosses of amicronucleates by micronucleates*

Crosses	No. prs.	Cytoplasmic exchange	Viability of exconjugants from: Amicronucleate mate V*	Amicronucleate mate N**	Micronucleate mate V	Micronucleate mate N
A. 51 VII killer amicronucleates ×138 XVI sensitives	23	+†	0	23	4	19(83%)
	6	−††	0	6	5	1
B. 138 XV sensitive amicronucleates ×51 VIII killers	2	+	0	2	2	0
	25	−	0	25	25	0

† + = cytoplasmic exchange.
†† − = no cytoplasmic exchange.
* V = viable.
** N = nonviable.

the death of the progeny of the stock 51 micronucleates.

The antiserum technique for induction of cytoplasmic exchange (Sonneborn, 1950) was used. Conjugating pairs from the intervarietal cross of amicronucleate stock 138 XV to micronucleate stock 51 killers and of the intravarietal cross of amicronucleate stock 138 XV sensitives to micronucleate 138 XVI sensitives were exposed to mixtures of antisera against the serotypes used in the cross. The exposure began five hours after the beginning of conjugation and continued for two hours. Then the pairs were transferred to fresh culture fluid and observed for cytoplasmic bridges.

A greater proportion of the stock 51 micronucleate exconjugants of the cross to stock 138 amicronucleates died (table 7, cross B) following induction of cytoplasmic exchange than died when cytoplasms were not exchanged (table 5, cross B); 87% as compared to only 8%. Although these results appear to confirm the assumption that introduction of foreign cytoplasm into the micronucleate causes death, the point is somewhat obscured by the failure to carry out an important control for the action of the antiserum on viability. The only measure of the lethal effects of the antisera comes from the intravarietal control cross of stock 138 XV amicronucleates to stock 138 micronucleates (cross D, table 7). Taking into consideration this nonviability, the lethal effect of cytoplasmic exchange in the intervarietal cross is still significant. However, a more direct control of the action of the antiserum treatment on stock 51 micronucleates is needed. This could have been obtained from the induction of cytoplasmic exchange in the variety 4 intravarietal amicronucleate by micronucleate cross.

One exconjugant descended from an amicronucleate, survived the cross of stock 138 XV amicronucleates to stock 51 killers (table 7, cross B). Its micronucleate mate died. This survivor will be discussed below with the other survivors from the amicronucleates.

The reason for the high frequency of cytoplasmic exchange in one of the intervarietal crosses and not in the other is not known. Perhaps the two amicronucleates used differed in their tendency to form cytoplasmic bridges at conjugation.

Cytological evidence for the detrimental action of the cytoplasm

Cytological corroboration of the detrimental action of foreign cytoplasm was obtained from the following experiment. The four crosses listed in table 8 were made. Pairs from each cross were put into separate mass cultures and allowed to complete conjugation together. Other pairs from these same crosses were isolated individually and examined for via-

TABLE 7. *The effect of the induction of cytoplasmic exchange by the antiserum technique, on the viability of the exconjugants of the intervarietal and intravarietal crosses involving stock 138 amicronucleates*

Crosses	No. prs.	Viability of exconjugants from: Amicronucleate mate V*	Amicronucleate mate N**	Micronucleate mate V	Micronucleate mate N
B. 138 XV sensitive amicronucleates × 51 VIII sensitives	53	1(2%)	52(98%)	7(13%)	46(87%)
D. 138 XV sensitive amicronucleates × 138 XVI sensitives	36	6(17%)	30(83%)	20(56%)	16(44%)

* V = viable.
** N = nonviable.

TABLE 8. *Frequency of cytological abnormality six hours after the completion of conjugation and of nonviability in the exconjugant clones of the intervarietal and intravarietal crosses of amicronucleates to micronucleates*

Cytological abnormality is measured by the absence of micronuclei and macronuclei.

Crosses	Cytology		Viability of exconjugants		
	Normal	Abnormal	No. prs.	V*	N**
A. 51 VII killer amicronucleates × 138 XVI sensitives	15(17%)	74(83%)	30	6(10%)	54(90%)
B. 138 XV sensitive amicronucleates × 51 VIII killers	43(50%)	43(50%)	30	26(43%)	34(57%)
C. 51 VII killer amicronucleates × 51 VIII killers	31(65%)	17(35%)	30	33(55%)	27(45%)
D. 138 XV sensitive amicronucleates × 138 XVI sensitives			30	47(78%)	13(22%)

* V = viable.
** N = nonviable.

bility. Six hours after separation of the conjugants, all the animals in each mass culture were fixed and stained. (The control intravarietal cross of stock 138 amicronucleates to micronucleates was not stained.) Thus it was possible to compare the cytological picture with the viability of the exconjugants of three crosses.

Six hours after conjugation, the macronuclei of normal exconjugants should be broken down into fragments and the products of the syncaryon should be differentiated into micronuclei and macronuclear anlagen. At the start of the matings one member of each pair was a normal animal, so it might be expected that at least one-half the exconjugants would show the normal cytological picture. The results of the experiment are summarized in table 8.

There was very close agreement between the percentages of exconjugants which were cytologically abnormal (as measured by the absence of micronuclei and macronuclear anlagen) and the percentages of nonviable exconjugant clones on the one hand, and cytologically normal exconjugants and viable clones on the other hand. Thus in the intravarietal cross of the stock 51 amicronucleates to stock 51 micronucleates (table 8, cross C) some of the amicronucleate conjugants must have received micronuclei from their mates, since 65% of the exconjugants stained were normal, and 55% of the exconjugants (three descended from the amicronucleate conjugants) survived. The intervarietal cross of stock 138 amicronucleates to stock 51 killers (table 8, cross B) was the cross in which cytoplasmic exchange did not naturally occur in high frequency (table 6, cross B) and in which 92% of the micronucleates regularly survived (table 5, cross B). As might be expected, half the exconjugants of this cross showed the normal cytological picture which was correlated with the survival of almost all of the micronucleates. The cytologically abnormal exconjugants must have been derived from the amicronucleates, none of which survived. In the cross of stock 51 killer amicronucleates to stock 138 (table 8, cross A) only 17% of the exconjugants were cytologically normal and these could be correlated with the 10% survival resulting from the cross. All the survivors were descended from the micronucleates. Since 50% of the conjugants of this cross

originally had micronuclei, it follows that nuclei were *destroyed* in some of the micronucleates. This is the cross in which cytoplasmic exchange occurred in high frequency and in which most of the progeny of the micronucleate conjugants died (table 6, cross A).

The discoveries made using the amicronucleates are really striking. Not only are small amounts of cytoplasm found to be effective in causing death of the micronucleates in the intervarietal crosses (exchanges of cytoplasms lasting only two to three minutes are effective), but such small amounts are not known to affect the other traits which are sensitive to cytoplasmic exchange, such as the killer, mate-killer, antigen and mating type traits. The action of the cytoplasm must be both drastic and rapid, since nuclei are destroyed within six hours after cytoplasmic exchange is completed.

The death of the vast majority of the amicronucleates in the intervarietal crosses must now be discussed. That regular introduction of foreign genes into the amicronucleates takes place has not been established. As had been mentioned, staining six hours after the completion of conjugation failed to show micronuclei. This may be because the foreign micronuclei, although they entered, were quickly destroyed. Another possible interpretation is that the amicronucleates could not be readily fertilized by the foreign nuclei. Staining the conjugants for micronuclei immediately after the fertilization stage takes place, should shed light on this problem.

Summary and conclusions

Hybrid genomes cannot be formed in the intervarietal crosses of amicronucleates to micronucleates, yet mortality occurs. A correlation has been established between the introduction of cytoplasm from the amicronucleates of one variety into micronucleates of the other variety and the destruction of the nuclei followed by the death of the micronucleates. Thus the F1 mortality in the cross of variety 4 to 8 must be primarily due to a system of nucleo-cytoplasmic incompatibilities, rather than to an intranuclear incompatibility. As the cytoplasms of both varieties are effective against foreign nuclei, there must be at least two cytoplasmic components to the system, one for each variety. It is not clear whether the action of the foreign cytoplasm is directly on the nucleus of the other variety or whether the two cytoplasms interact first and then affect the nuclei. Against the idea that the cytoplasms must first interact is the death of the F1 in the intervarietal micronucleate to micronucleate crosses, even when no cytoplasmic exchange is noted. However, it can always be supposed that enough cytoplasm adheres to the migratory micronuclei to set up the lethal interaction.

Some findings by Lorch and Danielli (1950) might also be interpreted as due to a nucleo-cytoplasmic incompatibility. These authors have devised a technique for transferring nuclei of amebae from one cell to another. About 65% of the animals survived when nuclei were transferred from one animal to an enucleated animal of the same species. However, when reciprocal transfers were made between cells of different species, the nucleus of each species failed to function in the enucleated cytoplasm of the other species and the cells died in all but one case out of 121. The death of these latter cells may be caused by a lethal interaction of nucleus and foreign cytoplasm. It would be of interest to know what happens when nuclei of both species are placed together in the same cytoplasm.

The Significance of the Survivors Descended from the Amicronucleate Conjugants of the Intervarietal Crosses

Four viable clones were found which could have been derived from the amicronucleate conjugants of the intervarietal crosses. What is the nature of these animals? Two of these four are cases of

the transformation of the progeny of variety 8 amicronucleates into normal variety 4 animals. The other two viable cultures may be cases of the transformation of variety 4 amicronucleates into normal variety 8 animals; but in these two cases, the interpretation is not so clear.

The first case of the survival of an exconjugant clone derived from the amicronucleate occurred in the cross of stock 138 XV sensitive amicronucleates to stock 51 VIII killers (table 5, cross B). Both members of one pair were viable. One of the exconjugants passed through 10 fissions four days after conjugation was completed, while the other exconjugant took six days to complete the same number of fissions. The more rapidly dividing exconjugant undoubtedly was derived from the killer conjugant as it gave rise to a killer clone of the even mating type. The more slowly dividing exconjugant produced a sensitive clone, also of the even mating type. The sensitivity of this clone marked it as cytoplasmically derived from the stock 138 amicronucleate. Further, clones derived from the amicronucleates usually divide at first more slowly than those derived from their micronucleate mates. Added evidence that these two clones were from the cross of an amicronucleate to a micronucleate came from the mating type of the survivors. The change of mating type of the sensitive clone from odd to even is the observed result of mating an amicronucleate animal of the odd type to a micronucleate animal of the even type (Sonneborn, unpublished, for variety 4; Levine and Siegel, unpublished, for variety 8).

These clones showed extreme nonviability when allowed to undergo autogamy in mass culture. After a time these cultures were mated to stock 51 killers and to stock 138 sensitives and both cultures showed high viability in the cross to stock 51 and low viability in the cross to stock 138. In other words, both cultures were now variety 4 animals, one was a mating type VIII killer and the other a mating type VIII sensitive.

The following interpretation is placed on these results. It is assumed that the migratory micronucleus from the micronucleate stock 51 killer conjugant entered the stock 138 amicronucleate and was able to function, the mating producing two haploid exconjugant clones. These clones then underwent autogamy, resulting in the death of most of the animals. A few cases of restitution of the diploid chromosome number must have taken place to produce a few diploid variety 4 animals in both cultures, which then predominated. Thus the variety 8 animal was transformed into a normal variety 4 animal.

A similar interpretation is placed on a survivor of the cross in which cytoplasmic exchange was induced in the mating of stock 138 XV sensitive amicronucleates to stock 51 VIII killers (table 7, cross B). One member of the pair died and the other completed 10 fissions on the sixth day after conjugation. The surviving clone was a sensitive of the even mating type, indicating that it came from the amicronucleate member of the pair. The clone selfed and the progeny were nonviable. This mortality would be expected of a haploid clone. This clone was also allowed to undergo mass autogamy and eventually became a normal variety 4 sensitive culture.

The other two cases of the survival of both members of a pair occurred in the cross of stock 51 killer amicronucleates to stock 130 XVI sensitives (table 6, cross A). However, it is not clear that these are cases of the transformation of variety 4 amicronucleates into variety 8 animals, because the killer trait used to identify the progeny of the variety 4 conjugants was lost. It is possible, therefore, that these survivors may originally have been descended from variety 8. Had one exconjugant of each of these pairs been marked with the killer trait, there would be no question that the variety 4 amicronucleates had been transformed into variety 8 animals. It might be assumed that the transformation had occurred and that killing was not found because the genes

of stock 130 cannot support the killer trait. Thus the question of transformation of the animals of one variety into those of the other in these two cases must be left undecided.

In two cases the genes of variety 4 were successfully introduced into variety 8 animals and were able to function in foreign cytoplasm, eventually giving rise to normal variety 4 cultures. These facts appear to be in striking contradiction to the evidence presented for the lethal interaction of the cytoplasm of each of the two varieties with the genes of the other. However, the survival of these transformed cultures is considered further evidence of the lability of the substances involved in the lethal intervarietal reaction.

General Discussion

The isolation of varieties 4 and 8

These studies confirm previous work (Sonneborn and Dippell, 1946b; Melvin, 1949) which established the genetic isolation of varieties 4 and 8. The mortality resulting from intervarietal crosses is of special interest since animals of the two varieties are found in nature in close proximity to each other. The observed mortality necessarily results in a high degree of genetic integrity for the two varieties.

The genetic control of the incompatibility substances

The physiological incompatibility of the nuclei of each variety with the cytoplasm of the other, preventing as it does the free exchange of genetic material, might be interpreted according to the suggestion of Jones (1951) that cytoplasmic differentiation may be a prerequisite for the final separation of species. It is not clear, as Jones recognized, whether the cytoplasmic components of such a system are initiated by the genes or whether they have a measure of independence. The transformation of variety 8 amicronucleates into animals possessing the variety 4 incompatibility substances argues strongly for the initiation of these substances by the genes. There is, however, no evidence against the argument that the newly acquired incompatibility of these transformed animals may be due to the introduction of these substances with the variety 4 migratory micronucleus. A decisive test for these alternatives would be very important.

A biochemical analysis of the morbid interaction of nucleus and cytoplasm in the intervarietal crosses is intended. Such a study, if successful, would not only elucidate the mechanism of intervarietal death, but might also produce important information about the destruction of genetic material. Also, a method of eliminating the lethality in the intervarietal crosses may result from such an analysis. This would make interspecific genetics possible.

Phylogeny

Any attempt at setting up a phylogeny of the varieties of *Paramecium aurelia* at the present time would be no more than guess work. Information about chromosome number and morphology, frequency of many genetic traits, ecological requirements and other factors concerning the different varieties is lacking. However, one is tempted to speculate that the close association of varieties 4 and 8 in nature is a manifestation of their common origin. It may, of course, only be indicative of the similar but more specialized ecological requirements of variety 8. The inability of most of the animals in the mixtures of mating types VII with XVI to complete conjugation may be a foreshadowing of the eventual sexual isolation of the two varieties.

Summary

An examination of the intervarietal breeding relations of all the stocks of varieties 4 and 8 of *Paramecium aurelia* demonstrates the genetic isolation of these varieties. The F1 generation resulting from the intervarietal crosses is 95% nonviable. Analysis shows some of the survivors of these crosses to be nonhybrid,

leaving the nature of the others in a state of uncertainty.

Crosses between amicronucleate animals of each variety and normal animals of the other demonstrates that the extreme nonviability of the F1 generation is due to the incompatibility of the cytoplasm of each variety with the nucleus of the other. Evidence for controlling the nonviability is presented. The transformation of amicronucleate animals of one variety into normal animals of the other has been accomplished in a few cases.

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Literature Cited

Butzel, H. M., Jr. 1953. Production of amicronucleate lines of *Paramecium aurelia* by colchicine treatment. Microbial Genetics Bull., **7**: 32.

Chen, T. T. 1946a. Conjugation in *Paramecium bursaria*. I. Conjugation of three animals. J. Morph., **78**: 353–396.

——. 1946b. Conjugation in *Paramecium bursaria*. II. Nuclear phenomena in lethal conjugation between varieties. J. Morph., **79**: 125–262.

Dippell, R. V. 1950. Mutation of the killer cytoplasmic factor in *Paramecium aurelia*. Heredity, **4**: 165–187.

Jones, D. F. 1951. The cytoplasmic separation of species. Proc. Nat. Acad. Sci., **37**: 408–410.

Levine, M. 1953. The diverse mate-killers of *Paramecium aurelia*, variety 8: their interrelations and genetic basis. Genetics, in press.

Lorch, I. J., and J. K. Danielli. 1950. The transplantation of nuclei from cell to cell. Nature, **166**: 329–330.

Melvin, J. B. 1949. Hybrid mortality in intervarietal crosses in *Paramecium aurelia*. Master's Thesis, Wellesley College.

Nanney, D. L. 1953. Mating type determination in *Paramecium aurelia*, a model of nucleo-cytoplasmic interaction. Proc. Nat. Acad. Sci., **39**: 113–119.

Preer, J. R. 1948. A study of some properties of the cytoplasmic factor, "Kappa," in *Paramecium aurelia*. Genetics, **33**: 349–404.

——. 1950. Microscopically visible bodies in the cytoplasm of the "killer" strains of *Paramecium aurelia*. Genetics, **35**: 344–362.

Siegel, R. W. 1953a. Mate-killing in *Paramecium aurelia*, variety 8. Phys. Zool., in press.

——. 1953b. A genetic analysis of the mate-killing trait in *Paramecium aurelia*, variety 8. Genetics, in press.

Sonneborn, T. M. 1940. The relation of macronuclear regeneration in *Paramecium aurelia* to macronuclear structure, amitosis, and genetic determination. Anat. Rec., **78** (suppl.): 53–54.

——. 1942. More mating types and varieties in *Paramecium aurelia*. Anat. Rec., **84**: 542.

——. 1943. Gene and cytoplasm. I. The determination and inheritance of the killer character in variety 4 of *Paramecium aurelia*. II. The bearing of the determination and inheritance of the characters in *Paramecium aurelia* on the problems of cytoplasmic inheritance, pneumococcus transformations, mutations and development. Proc. Nat. Acad. Sci., **29**: 329–343.

——. 1944. Exchange of cytoplasm at conjugation in *P. aurelia*, variety 4. Anat. Rec., **89**: 576.

——. 1947. Recent advances in the genetics of Paramecium and Euplotes. Advances in Genetics, **1**: 263–358.

——. 1950. Methods in the general biology and genetics of *Paramecium aurelia*. J. Exp. Zool., **113**: 87–148.

Sonneborn, T. M., and R. V. Dippell. 1946a. Mating reactions and conjugation between varieties of *Paramecium aurelia* in relation to conceptions of mating type and variety. Phys. Zool., **19**: 1–18.

——. 1946b. The significance of race 31 as a link between group A and B varieties of *P. aurelia*. Anat. Rec., **96**: 515.

Sonneborn, T. M., and A. LeSuer. 1948. Antigenic characters in *Paramecium aurelia* (variety 4): determination, inheritance and induced mutations. Amer. Nat., **82**: 69–78.

NOTES AND COMMENTS

THE "BALDWIN EFFECT," "GENETIC ASSIMILATION" AND "HOMEOSTASIS"

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When preparing my article on "Genetic Assimilation of an Acquired Character,"[1] I debated with myself whether to include a discussion of the old train of thought usually referred to as "organic selection," but in the interests of brevity decided not to. However, the immediately preceding article by Simpson on "The Baldwin Effect"[2] dealt with just this subject, and it would now perhaps be useful to indicate the way in which the idea which I was putting forward differs from those which he recapitulated.

Simpson describes the Baldwin Effect as taking place in three stages, which put very shortly are: (1) Individual organisms interact with the environment in such a way as to produce non-hereditary adaptations; (2) Genetic factors producing similar traits occur in the population; (3) These factors increase in frequency under natural selection. The gap in the argument is between steps (1) and (2). Is there supposed to be any connection between the developmental adaptations and the genes with similar effect, and if so, what? Simpson (p. 115) says that either there is no particular connection, in which case the theory signifies very little, or the connection must be by way of a neo-Lamarckian causal connection. Huxley (Evolution: The Modern Synthesis, 1942, p. 304) seems to put the point originally made by Baldwin and Lloyd Morgan more clearly when he writes that the adaptive modifications operate "by holding the strain in an environment where mutations tending in the same direction will be selected." Thus according to both Simpson and Huxley, the theory of "organic selection" still leaves the actual nature of the adaptive changes produced to the operation of random mutations or Lamarckism.

The theory which I put forward in 1942, in an article which Simpson seems to have overlooked[3] (Nature, Vol. 150, p. 563), and which provided the basis for the experiments described in my Evolution paper, attempted to go considerably further. I argued that natural selection for the ability to produce an adaptive phenotype would change the genotypes in such a way as to encourage the appearance of genetically controlled variants mimicking the adaptive type. The initial non-hereditary response therefore does not merely allow the organism to persist in a new environment and become adapted to it; it enables natural selection to set the stage in such a way that the useful genetic effect is likely to occur.

Simpson comes to the conclusion that the Baldwin effect, in the sense he describes it, has probably played a rather small role in evolution. The genetic assimilation mechanism, however, must be a factor in all natural selection, since the properties with which that process is concerned are always phenotypic; properties, that is, which are the products of genotypes interacting with environments. By speaking of mutations as "random," which is true enough at the level of the gene as a protein-DNA complex, we obscure the fact that the effect of a mutation, as far as natural selection is concerned, is conditioned by the way it modifies the reaction with the environment of a genotype which has already been selected on the basis of its response to that environment. This is not neo-Lamarckism, but it is a point which has been unduly neglected by neo-Darwinism.

The idea of the "canalization" or "buffering" of development (Waddington, 1939, 1940), which underlies the theory of genetic assimilation, is also closely related to the concept of homeostasis, which has recently been introduced in genetical theory. This word has been used in two senses, which should be distinguished. Lerner (1950) has applied it in connection with the tendency of the gene frequencies in a population, after disturbance by artificial selection, to return, under the influence of natural selection, to an equilibrium state. This may be called "homeostasis of gene ratios" or "genetic homeostasis." The aspect of natural selection concerned in maintaining it is what I have called "normalising selection" (Waddington, 1953). Dobzhansky and Wallace (1953) use the term in quite a different connection; an organism is said to be homeostatic when it "adjusts itself to recurrent environmental changes in such a

[1] Evolution, Vol. VII, No. 2, p. 118.

[2] *Ibid.*, p. 110.

[3] Schmalhausen in his "Factors of Evolution," 1947, meant, I think, either nearly or quite the same thing, but seems to confuse the "normalising" action of selection, which keeps a population uniform, with its "stabilising" action, which renders development relatively independent of environmental variations.

way that its function continues unimpaired." This might perhaps be called "developmental homeostasis." But the word homeostasis is perhaps an unfortunate one, since it seems to imply a stationary state, whereas development essentially involves change in time. It is for this reason that I have preferred the words canalization or buffering, which refer to an equilibrium sequence of states rather than to one unchanging equilibrium state. For the aspect of natural selection concerned with setting up such buffered developmental systems, I have recently (1953) used the term "stabilising selection," which was introduced by Schmalhausen (1949) who, however, does not clearly distinguish it from normalising selection. But again this is perhaps not a very satisfactory expression, since the stabilisation of development would seem to imply that change had been brought to a standstill. Probably the best expression would be "canalizing selection," derived from the word used in the original discussion of the concept.

References

Dobzhansky, T., and B. Wallace. 1953. Proc. Nat. Acad. Sci., **39**: 162.

Lerner, M. 1950. Population genetics and animal improvement. Cambridge Univ. Press.

Waddington, C. H. 1939. Introduction to modern genetics. London.

Waddington, C. H. 1940. Organisers and genes. Cambridge Univ. Press.

Waddington, C. H. 1942. Nature, **150**: 563.

Waddington, C. H. 1953. Symp. Soc. Exp. Biol., **7**: 186.

COMMENTS ON THE ORIGIN OF SEX AND OF MEIOSIS

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Boyden's (1953) paper on comparative evolution was most stimulating. His discussion of the origin of sexual reproduction is noteworthy for the emphasis upon the thesis that parthenogenesis is a modified form of sexual reproduction. He envisions the origin of sexual reproduction in terms of the origin of meiosis, but no theory is presented as to how or why meiosis originated beyond that it must have been a far simpler transformation than the previous development of mitosis. It is the purpose of the present note to add some further thoughts upon these problems.

As Boyden points out, terms like haploid and diploid have no meaning in relation to organisms at the pre-mitotic level of organization. The ability of bacteria to reproduce by simple fission, without any orderly alignment and segregation of the chromatin, indicates that the relatively few kinds of genes which they possess are probably all duplicated several times and scattered at random through the cell, so that both daughter cells are almost certain to contain genes of every kind. These might be called *multigenes*. But once the mitotic mechanism was established, haploidy must have been the norm for mitotically reproducing organisms. This is indicated by the lack of logical meaning of diploidy for an asexual organism (polyploidy would seem more reasonable for organisms derived from precursors with multigenes); by the frequency of odd numbers of chromosomes among asexually reproducing algae and Protozoa; and by certain facts relative to sexual reproduction in the algae, to be recounted below.

It is difficult to visualize why successful asexual organisms should ever have united pairwise to reproduce sexually. Because gametes of many algae appear to be simply small sized spores, the hunger theory of sex has been proposed, according to which the gametes are so small that, individually, they lack the minimum nutritive requirements for successful development; and these subminimal cells pool their resources by means of two-by-two fusions. The theory would be more satisfying were it not that the first divisions of a zygote are the two meiotic divisions, producing a group of four zoospores which successfully develop to maturity, in spite of their small initial size. Coulter (1914) examined and rejected this theory nearly forty years ago, but in its stead he was only able to suggest that sexuality was associated with metabolic products of a waning metabolism, somehow associated with the reduction divisions. He arrayed an impressive argument, but the key substances remained hypothetical. So we are left with no satisfactory explanation of the origin of sex, but once it did originate, great selective value made it nearly universal. Its selective value lies chiefly in the rapid reshuffling of character combinations, which submits a maximum number of phenotypes to the test of natural selection in a brief time. Thus new adaptive combinations are more likely to be hit upon than with asexual reproduction.

The origin of meiosis is perhaps a simpler problem than the origin of sex. We have clues

in the facts (1) that diploidy is the only possible result of the fusion of two haploid organisms (gametes); and (2) that in those algae which are the most primitive sexually reproducing organisms known (or should some bacteria have this position?) the meiotic divisions occur in the zygote, so that the zygote is the only diploid cell in the entire life cycle (Fuller & Tippo, 1949). Thus it appears that diploidy was introduced as a more or less accidental concomitant of sexual reproduction, and the meiotic divisions were developed as a means of restoring the normal, functional, haploid chromosome number. But there is also a real advantage to diploidy: it doubles the amount of nuclear material under the control of which the major reactions of the cell occur. Also it makes possible the accumulation of hidden variability in the heterozygous state. For these reasons, selection favored the further development of the diploid phase. In the Animal Kingdom, diploidy has become very nearly universal. In the Plant Kingdom, there is an alternation of a haploid sexually reproducing generation (gametophyte) and a diploid, asexually reproducing generation (sporophyte). In very primitive algae like *Chlamydomonas*, the haploid generation predominates heavily; in *Ulva*, the two generations are about equally developed; while in the land plants there has been a progressive quantitative increase in the importance of the sporophyte and a corresponding decrease in the importance of the gametophyte. This has culminated in the Angiosperms, in which the gametophyte is so much reduced that it would almost certainly be regarded as simply an organ of the sporophyte, were not comparative material from lower plants available.

In summary, then, it may be said that no satisfactory theory is available to explain the origin of sex; that the mitotically reproducing pre-sexual organisms were haploid; that diploidy originated as a necessary consequence of the fusion of two haploid gametes; that meiosis originated not as a step in gamete formation but as a means of restoring the primitive, functional haploid state of the organism; and finally that selection favored further development of the diploid phase of the life cycle because of the advantages of an increased amount of nuclear material and of the accumulation of variability in the heterozygous state.

Literature Cited

Boyden, Alan A. 1953. Comparative evolution with special reference to primitive mechanisms. Evolution, **7**: 21–30.

Coulter, John M. 1914. Evolution of sex in plants. University of Chicago Press, Chicago.

Fuller, Harry J., and Oswald Tippo. 1949. College botany. Henry Holt & Co., New York.

THE SEX CHROMOSOME OF THE HONEY BEE

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The actual existence of a sex chromosome in the honey bee, first discussed by Manning (1949), has recently been challenged in this Journal by Ris and Kerr (1952). Fortunately the challenge itself had previously been met (Manning, 1952), as a result of the use Mackensen (1952) made of Ris and Kerr's then unpublished work. The essential point raised by Ris was that the Feulgen technique was not believed to reveal the presence of an X-chromosome and that, in consequence, the "body" revealed by other techniques was presumed to be a nucleolus. However, apart from the difficulty of obtaining good results with honey bee spermatocytes by the use of such a technique, it is clearly quite unsuitable for revealing chromosomes with abnormal chromatic cycles. This applies to the X-chromosome of the honey bee in which a negative heteropycnosis can be demonstrated. If Ris and Kerr thus persist in this line of argument, it will be incumbent on them to disprove the negative heteropycnosis. Fortunately, photographic evidence of this phenomenon has now been accumulated though it is difficult to publish satisfactorily on account of the limited contrast.

We may now question other cytological matters discussed by them. The first is the precise nature of their observations at second spermatocyte metaphase. The point raised here is that a side-view—a usual configuration for observing the differentially behaving "body" which at this stage is demonstrably separating from the main chromosome group—does not permit accurate counts of the chromosomes themselves. On the other hand, a polar view does permit such counts and Ris and Kerr claim to have seen "16 feulgen positive chromosomes including the large hook-shaped one" in addition to the differential "body" which, of course, is still within

the spindle. This claim certainly calls for very careful examination: it means that Ris and Kerr claim to have recognised 17 "bodies," a number never mentioned at this stage by any previous worker.

We must now return to the question of the interpretation of the differentially behaving "body" as a nucleolus. First we must ask of Ris its precise nature, persisting as it does from "the prophase of the primary spermatocyte into the spermatid," in view of the fact that its chromatisation can be shown to be greatest at prometaphase and least at the telophases. Second, since the body is actually a dual structure why have these not fused as would frequently happen with nucleoli? To put this conversely, why is this body always dual in structure like the remaining chromosomes? Third, since this "nucleolus" can be demonstrated with spindle fibres attached to it (fig. 1, no. 1), to what peculiarity of the nucleolus can such fibres be attributed? Fourth, a study of vespoid wasps reveals the presence of a supplementary spindle that actually controls the behaviour of these bodies (fig. 1, no. 2). Why should such a spindle develop in connection with a nucleolus?

We must now ask ourselves a further question: What is the nature of this "nucleolus" at oocyte maturation? A glance at figure 1, no. 3 shows a metaphase of the primary oocyte in which a chromosome lies out of alignment with the other chromosomes. This is the sole differentially behaving "body." In this case it is unquestionably a chromosome which is late reaching the plate. The delay is likely on several grounds but particularly in connection with the retention of the chromosome for the secondary oocyte. If then such a differential

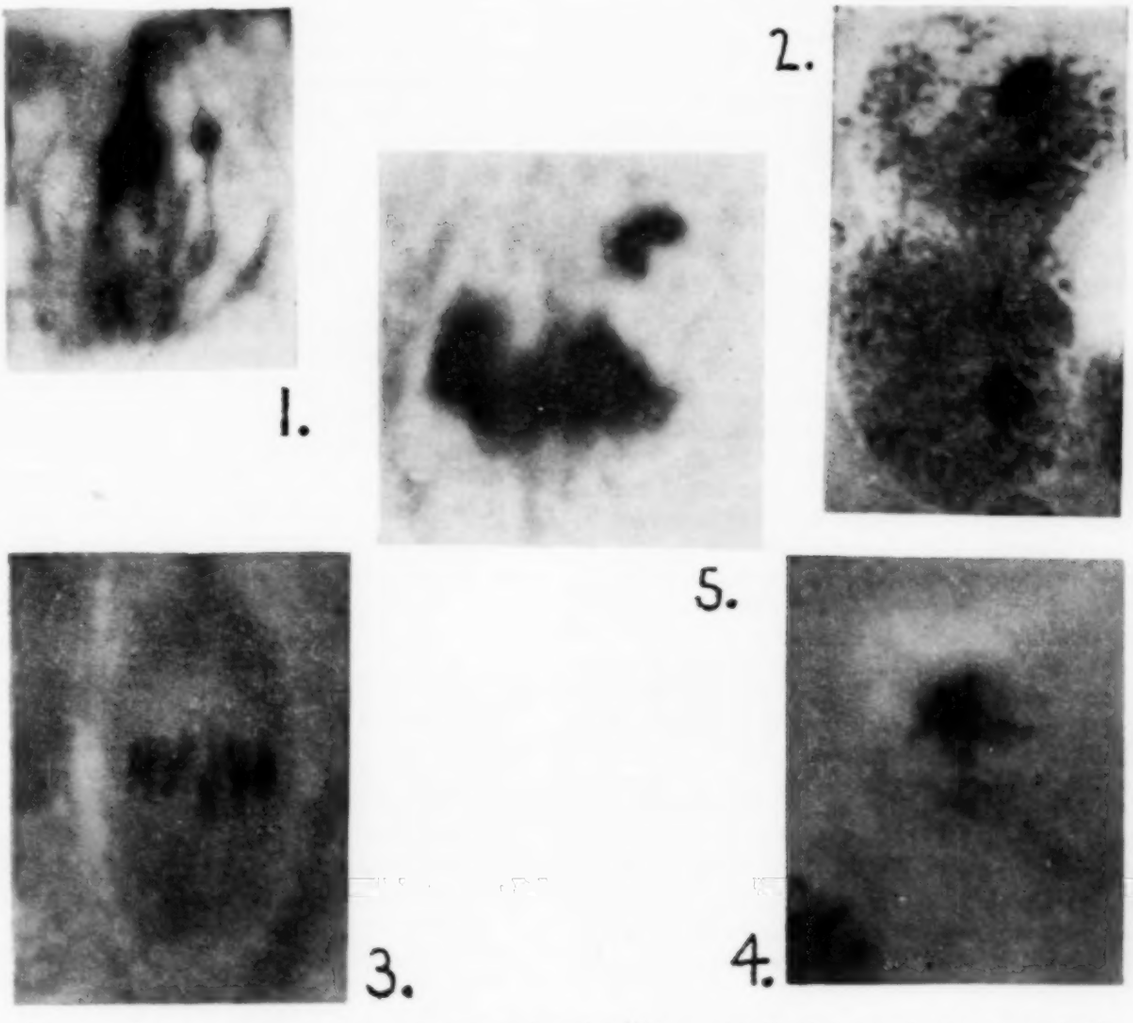

FIG. 1. 1. Spermatocyte, honey bee, showing the differential "body" attached to its spindle fibres. 2. Pro-spermatids, *V. germanica*, showing supplementary spindle between the two small, supernumerary nuclei; main nuclei larger and more distinct in daughter cells. 3. Oocyte, honey bee, metaphase 1, showing one chromosome late in reaching the plate. 4. First cleavage, honey bee; X-chromosome, delayed in its movement with the autosomes, lies detached at 6 o'clock. 5. Spermatocyte, *B. terrestris*, metaphase 2, showing "dyad" structure of the differentially behaving "body."

FIG. 2. 1. Oogonial metaphase plate. Large autosomes, 10 o'clock, single X-chromosome, 1 o'clock. 2. Second spermatocyte metaphase showing two large chromosomes only, at 7 o'clock and 1 o'clock respectively.

chromosome exists here why must we assume that the differential body in the male is a nucleolus? The actual shape of this body is very fortunately sometimes revealed at first cleavage owing to the somewhat greater delay of its movement in relation to the autosomes. Figure 1, no. 4 clearly indicates its "hooked" character. Moreover, the reader will recall that a slight delay of this chromosome is apparent in many somatic cells but is never sufficient to upset the equational character of the metaphases. It has already been demonstrated in late cleavage and blastoderm cells (Manning, 1952).

Proof of a similar state of affairs can be found among other members of the Hymenoptera. It is possible here to consider only a single aspect of the bumble bee, *Bombus terrestris* (L.). Its spermatogenesis is remarkably reminescent of the honey bee (cf. fig. 1, no. 5). Of the 16 chromosomes of the haploid set (fig. 2, no. 2) only two, however, are large. Nevertheless diploid oogonial plates (fig. 2, no. 1) reveal two large chromosomes associating together and one single chromosome lying separate from them. In such occurrence of a differential oocyte maturation and the existence of a single X-chromosome.

Finally, a point remains which, though not bearing on Ris and Kerr's article, is relevant to the questions raised here. It has been pointed out (Mackensen, *op. cit.*) that the gynandromorphs studied by Rothenbuhler *et alii* (1951, 1952) do not support the theory of genic balance, based on the X-chromosome, as the mechanism of sex determination in the honey bee (Manning, *op. cit.*). The argument is that supernumerary sperms, presumably producing the sex mosaics, must be androgenetic and, therefore, on Manning's hypothesis do not carry an X-chromosome and must thus be inviable. It is unfortunate Mackensen forgot he was dealing with abnormalities and therefore did not indicate how on his theory potentially viable sperm (i.e., possessing 16 chromosomes) do in fact become viable in the ooplasm only on those rare occasions when such gynandromorphs are produced. I suggest that a much more promising line of argument is to look for irregularities at spermatogenesis where certain sperms may abnormally retain the X-chromosome. In the ooplasm they may be capable of division to an extent sufficient to effect sex mosaics, whereas a normal sperm (i.e. containing no X-chromosome) is completely inviable unless it first fuses with the egg pronucleus.

This article contains a number of points as yet inadequately discussed in the literature. Fortunately the preliminary statement made by Manning (1949–50) will soon be replaced by a main article dealing with these matters. I wish finally to thank Mrs. Bisbee, University of Liverpool, for the very generous way she has given of her time to checking my material, and also for her constant encouragement.

LITERATURE CITED

MACKENSEN, O. (AND F. RUTTNER). 1952. Bee World, **33**(5): 71–79.

MANNING, F. J. 1949–50. Microscope, **7**: 175, 209, 237, 259, 303, 329; **8**: 6, 63, 129.

——. 1952. Microscope, **9**: 93–98.

RIS, H., AND W. E. KERR. 1952. Evolution, **6**(4): 444.

ROTHENBUHLER, W. C., J. W. GOWEN, AND O. W. PARK. 1951. Genetics, **36**(5): 573.

——————. 1952. Science, **115**: 637–638.

THE EVOLUTION OF MATING BEHAVIOR PATTERNS[1]

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Since it was recognized that instinctive behavior patterns often are, as taxonomic characters, as reliable as are morphological structures, increased attention has been given to comparative study of behavior as a preliminary to evolutionary conclusions. Mating behavior has an additional interest since, when it acts as an isolation mechanism, there is a premium on interspecific diversity, at least in sympatric species. *Drosophila*, rich in species and genetically well explored, appears to be an excellent object for such studies, as Mayr has pointed out repeatedly.

Expanding the work of Sturtevant, Mayr and others, Spieth observed the normal mating behavior of 101 species and subspecies in 21 species groups of the genus *Drosophila*. Definitions of terms of distinct elements of mating behavior, thought to be roughly homologous throughout the genus, are given. The descriptions that follow are mainly qualitative, though some quantitative data are also given.

Of the eight recognized subgenera, three of the larger ones (*Pholadoris*, *Sophophora* and *Drosophila*) have been extensively studied. On the whole, the behavior is remarkably uniform, and it is therefore concluded that the divergence in mating behavior must have occurred "at the sensory and psychological levels rather than at the mechanical and physical action levels." The behavior data tally well with the taxonomy based on other characters.

Those actions that are observed in many modern species are taken to have occurred in the ancestral form. On this assumption a tentative sketch of the mating behavior of the ancestral form is given, and its subsequent radiation is discussed. The differences between *Sophophora* and *Drosophila* are considered in detail; *Sophophora* has specialized in distance stimulation and reception, *Drosophila* in contact signals.

It is interesting to compare these findings with what little is known of other groups. Anatidae show, even within genera, on the whole much wider divergences in motor patterns, both visual and auditory, and in the visual sphere also in structural releasers. The genus *Larus* has developed striking differences in calls and visual releasers, both structural and behavioral. Nevertheless, as in *Drosophila*, differences between very closely related species seem to have evolved more easily in thresholds and sensory mechanisms than in motor patterns. *Uca* shows enormous radiation in movements and structure of the claw.

This paper is an invaluable basis for further work. An important task will be the analysis of the motivation underlying the behavior in order to distinguish between simpler and compound (ambivalent) movements, which again is a prerequisite for a sound judgment of homology. This will be especially urgent in groups which have undergone more extensive radiation of motor elements than *Drosophila*.

[1] Spieth, H. T. 1952. Mating behavior within the genus *Drosophila* (Diptera). Bull. Amer. Mus. Nat. Hist., **99**: 395–474.

COMMENT ON "CAVE FISH EVOLUTION"

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Recently C. M. Breder published in these columns[1] a comment on my paper[2] on *Caecobarbus geertsi*, a blind African cave fish. This comment cannot be considered as a fair account either of the facts presented, or of the hypotheses derived from them on cave evolution, and, as such appears to be liable to spread erroneous opinions among the readers of *Evolution*. Indeed, Breder fails to summarize the most fundamental of my statements in both spheres—facts and theories—and this omission only seems to confer on his criticism an appearance of soundness.

For what concerns my statements of fact, some very important ones are presented as if they were only extrapolations. For instance, the low metabolic rate of the fish was certainly presumed on the basis of morphologic features, but Breder does not mention that this was largely confirmed by actual measurements of the standard oxygen metabolism by H. J. Koch,

[1] Evolution, **7**: 179–181 (1953).

[2] Ann. Soc. Roy. Zool. Belgique, **82**: 155–230 (1951).

as I state it. On the other hand, this fact reinforces my assumption of the very low growth rate of the fish, on the basis of scale readings. And again, this very low growth rate is an observational fact as well, showing up in the best artificial cave conditions, with or without food, during a period of two years. To be sure, I have no proof for my further assumption, that the periodic scale marks correspond to the evident seasonal, yearly, cycle of food import by the tropical rains into the caves. But Breder does not mention that, unless this last assumption is made, namely, that of a causal relationship between the scale marks and a rhythmic, yearly, event, the allometric differences among the various populations form a highly improbable and unextricable mess. Only if this causal relationship is assumed, and growth is admitted to be very slow, all the data can be fitted into a reasonable picture, and a given population can be described in terms of the others.

A certain number of other criticisms concern my hypotheses on the adaptation of *Caecobarbus* to its habitat, and on the evolutionary mechanisms at work in the production of cave fish in general. Again, these criticisms are iterated without even mentioning the basic framework of these hypotheses. Indeed, I find no trace of the following fundamental statements I have made: (1) cave fish evolution is the result of a *hereditary* change; (2) this change is *adaptive* to the cave environment; (3) as such it is caused by a selective factor, assumed to be, in the case considered, low amounts of food in a closed habitat; (4) this selective process, conferring to the fishes the universal advantage of low food requirements, is, however, limited to cave conditions, absence of light and presence of carbon dioxide included, by its physiological consequences of low metabolism and probable loss of internal stability.

Now, if I am talking about a *hereditary* deceleration of the growth rate, and an equally *genotypic*, secondary loss of the eyes as its consequence, I do not care in the least what hundreds of pond culturists might eventually tell me about *modificatory* effects of starvation on the phenotype of their fishes. Nor would I bother about the fact, that even after many generations of starving fish, they still do not obtain anything resembling a cave fish, because a pond does not resemble a subterranean lake in any respect. A starved mouse does not resemble a hereditary pituitary dwarf, neither morphologically, nor certainly physiologically. This difference would show up especially with a limited food supply during growth. The pituitary dwarf would seem adapted to this minimal supply, because of the *genotypic* nature of its growth retardation. And, due exactly to this fundamental difference between genotypic and phenotypic variation, which still, after fifty years of genetics, it seems necessary to stress, the minimal food supply will be adequate for the dwarf, but inadequate for the normal mouse. Although I assumed that, in the case of African caves, the *originally* inadequate food supply acted as the effective selective factor, it is perfectly clear that no living being can be adapted to only one environmental factor. Cave fishes are necessarily adapted as well to the other conditions, differentiating a cave from a normal habitat. This is clearly expressed in my fourth basic assumption. This comes down to saying that in other caves, where more food is present, the effective selective factor might be darkness or CO_2 concentrations. The outcome, however, should be an adaptation to the whole complex of cave conditions, all of which seem to require and to allow a low standard metabolism. But I would not have any objection at all to cave fish with such a low standard metabolism eating eventually lots of food, if available, as seems to be the case in the Mexican cave fishes. I would not have an objection by virtue of another genetic principle, but one in fact not different from the first that I cited. This principle says, that the lability, the high degree of modifiability of a trait, does not exclude its hereditary determination. Although the standard metabolic rate of an animal is undoubtedly genotypically determined, its actual metabolism is one of the most highly modifiable "traits" one can imagine, under the influence of all kinds of conditions, and certainly in the presence of large amounts of food. Exactly the same can generally be said about overall or partial growth processes, especially in fishes. I would not have thought it necessary to restate that an organism can never be described as a stable system, but that it is always best characterized as a "steady state." A given positively allometric growth process, as I considered melanin deposition to be in early life, means therefore clearly, a temporary positive excedent of melanin deposition over its destruction. Nobody will doubt that given steady states of pigmentation are characteristic, and hereditary, for given species, although this equilibrium may be greatly changed by external conditions. In cave fishes, within their caves, the hereditary make up is such, that this equilibrium is practically at the zero level. This low level, I assumed to be *secondarily*, but *genotypically* determined, as a consequence of the *genotypical retardation* of overall physiological processes.

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